Please Do Not Reply To This Email.

Public Comments on Developing an Unified Intercarrier Compensation:========

Title: Developing an Unified Intercarrier Compensation
FR Document Number: 2011-04399
Legacy Document ID:
RIN:
Publish Date: 3/2/2011 12:00:00 AM

Submitter Info:
First Name: Felicia N
Last Name: Trujillo
Mailing Address: POB 28068
City: Santa Fe
Country: United States
State or Province: NM
Postal Code: 87592-8068
Organization Name: null

Your plan to deprive us of land lines and leave us with only cell phones will result in the deaths and illness of miillions of Americans. Truly such a plan smells of genocide.

CELL PHONES: Right now, the data shows a 500% rise in the rate of brain tumors, called gliomas, that no patient survives.

DISEASES: There is also a 360% rise in tumors of the eye nearest the ear used for the cell phone, and 26% rise in tumors on the hearing apparatus, and on salivary glands near the ear used for cell phones. This is all hitting the press now, but still in toned-down terms.

Although many studies are American funded and have American scientists, they are mostly being done in Europe and Canada to avoid exposure to Americans. It is like the Tobacco Industry all over again. There are 2000 journal published studies proving danger and the US Navy already performed 6000 studies when EMf was proposed as a method of mass destruction--only of humans--while retaining the infrastucture.

IF YOU TRY TO REMOVE LAND LINES, MANY WILL CONSIDER THAT AN ACT OF WAR TOWARDS THE AMERICAN PEOPLE.

Depriving us of land lines and causing the cancers and neurotoxic effects of low power electromagnetic radiation and microwave of the cell phones is a criminal activity and we will do everything in our power to stop this travesty.

Whatever corporate entity has entered into our government to foist this upon us must be revealed and expurgated.

The scientific community is well aware of not only cancers, but of the affects on hormones, cardiovascular function, the leakage of albumin from brain cells through the blood brain barrier, the sterility in men, the miscarriages in women--all caused by EMF.

There is no doubt in my mind and the mind of many educated persons that this is a plan to cause a massive depopulation and eventuate Big Brother in our lifetime. This

is a bipartisan issue and will be fought by both parties, and by all generations.

PATHOPHYSIOLOGY

Pathophysiology is an international journal publishing papers in English which address the etiology, development, and elimination of pathological processes. Contributions on the basic mechanisms underlying these processes, model systems and interdisciplinary approaches are strongly encouraged.

Pathophysiology has a broad scope and is managed by an international editorial board which consists of highly respected scientists from different speciality fields. As the biomedical sciences are becoming more interdisciplinary, *Pathophysiology* is eminently suitable for the publication of key articles in this field. Contributions covering the following areas are welcomed: general pathophysiological processes—inflammation, infection, hypoxia, stress, shock, pain, disregulation; cellular and molecular pathophysiology; neuroscience; heart and circulation; respiratory system; renal, fluid and electrolyte pathophysiology; gastrointestinal and hepatic system; endocrine system; metabolic disorders; blood system; immune system; reproductive system; locomotor system. The journal's audience comprises: physiologists, pathologists, physicians, neurologists, cardiologists, etc. in both university and hospital environments.

Pathophysiology is the official journal of the International Society for Pathophysiology. Members of this international society can enter a personal subscription to the journal at a special rate through the society. Membership enquiries should be addressed to the Secretary General of the ISP, Youyi Zhang, Institute of Vascular Medicine, Peking University Third Hospital No. 49, North Garden Road, Haidian District, Beijing 10083, China. Tel.: (+86) (10) 82802306. E-mail: zhangyy@bjmu.edu.cn

Publication information: *Pathophysiology* (ISSN 0928-4680). For 2009 volume 16 is scheduled for publication. Subscription prices are available upon request from the Publisher or from the Regional Sales Office nearest you or from this journal's website (http://www.elsevier.com/locate/pathophys). Further information is available on this journal and other Elsevier products through Elsevier's website: (http://www.elsevier.com). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request. Claims for missing issues should be made within six months of the date of dispatch.

Author enquiries: For enquiries relating to the submission of articles (including electronic submission where available) please visit this journal's homepage at http://www.elsevier.com/locate/pathophys. You can track accepted articles at http://www.elsevier.com/trackarticle and set up e-mail alerts to inform you of when an article's status has changed. Also acessible from here is information on copyright, frequently asked questions and more.

Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher.

Orders, claims, and journal enquiries: please contact the Customer Service Department at the Regional Sales Office nearest you:

Orlando: Elsevier, Customer Service Department, 6277 Sea Harbor Drive, Orlando, FL 32887-4800, USA; phone: (800) 6542452 [toll free within the USA]; fax (407) 3639661; phone: (+1) (407) 5636020 [outside the USA]; fax: (+1) (407) 3631354; e-mail: JournalsCustomerService-usa@elsevier.com

Amsterdam: Elsevier, Customer Service Department, PO Box 211, 1000 AE Amsterdam, The Netherlands; phone: (+31) (20) 4853757; fax: (+31) (20) 4853432; e-mail: JournalsCustomerServiceEMEA@elsevier.com

Tokyo: Elsevier, Customer Service Department, 4F Higashi-Azabu, 1-Chome Bldg, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044, Japan; phone: (+81) (3) 5561 5037; fax: (+81) (3) 5561 5047; e-mail: JournalsCustomerServiceJapan@elsevier.com

Singapore: Elsevier, Customer Service Department, 3 Killiney Road, #08-01 Winsland House I, Singapore 239519; phone: (+65) 63490222; fax: (+65) 67331510; e-mail: JournalsCustomerServiceAPAC@elsevier.com

ELSEVIER

Pathophysiology 16 (2009) 67–69

PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Preface

There is an old joke with a well-known punch line about a man who has just fallen from the 86th floor of the Empire State Building in New York. As he passes the 30th floor, he is heard saying to himself 'so far, so good'...

Most of us laugh because we know where the man is headed, and that he must know too. But, our laughter usually has a guilty edge. We know that many of us are guilty of occasionally displaying a 'so far, so good' attitude in our own lives. We think of the smoker who says that about the possibility of getting lung cancer or heart disease and who counts on beating the odds because he feels healthy at the moment. That smoker will not find out if he won the bet until many years later, and by then it is often too late. The 'so far, so good' attitude to health is so common that people even kid themselves about it. One smoker told me that smoking would only cut a few years off his life, and that he did not mind losing the last few years because they are usually not much fun anyway.

Unlike the optimist in the joke, whose end is virtually certain, many of us live like the smoker, playing the odds and reassuring ourselves 'so far, so good'. Diseases like cancer usually take many years to develop, and we try not to think how some of the things we do casually can affect the long-term odds by compromising the natural processes that protect us. We rely on our bodies to be strong and resilient all the time. Yet, we know there are limits to the body's natural ability to reverse damage to cells. We also know that there may be gaps in the ability of our genetic endowment to cope with damage. At some level, we all know it is just common sense to try to minimize damage to our bodies and maximize the ability to repair.

These opening paragraphs provide a quick introduction to the theme of this issue of Pathophysiology and a summary of the point of view of its authors. The public is currently interested in possible hazards from radio frequency (RF) due to cellphones, towers, WiFi, etc. The concern is certainly warranted, but we are surrounded by electromagnetic fields* (EMFs) of many frequencies, and there are also significant biological effects and known risks from low frequency EMF. The scientific problem is to determine the nature of EMF interaction with biological systems and develop ways of coping with harmful effects in all frequency ranges, as well as their cumulative effects. The practical problem is to minimize the harmful biological effects of all EMF.

The technical papers in this issue are devoted to an examination and an evaluation of evidence gathered by scientists regarding the effects of EMF, especially RF radiation, on living cells and on the health of human populations. The laboratory studies point to significant interactions of both power frequency and RF with cellular components, especially DNA. The epidemiological studies point to increased risk of developing certain cancers associated with long-term exposure to RF. Overall, the scientific evidence shows that the risk to health is significant, and that to deny it is like being in free-fall and thinking 'so far, so good'. We must recognize that there is a potential health problem, and that we must begin to deal with it responsibly as individuals and as a society.

Table of contents

EMF effects on DNA

M. Blank and R. Goodman (USA): Electromagnetic Fields Stress Living Cells

Abbreviations: EMF, electromagnetic fields; Hz, hertz (cycles/s the unit of frequency); ELF, extremely low frequency ($3–3 \times 10^3$ Hz) power frequency is 50–60 Hz; RF, radio frequency (band width 3×10^5 to 3×10^{11} Hz); UHF, ultrahigh frequency band the RF sub-division used for cell phones (3×10^8 to 3×10^9 Hz).

0928-4680/$ – see front matter © 2009 Published by Elsevier Ireland Ltd.
doi:10.1016/j.pathophys.2009.02.002

J.L. Phillips, N.P. Singh and H. Lai (USA): Electromagnetic Fields and DNA damage

H.W. Rüdiger (Austria): Genotoxic effects of electromagnetic exposure in vitro

EMF effects on the brain

H. Nittby, A. Brun, J. Eberhardt, L. Malmgren, B.R.R. Persson and L.G. Salford (Sweden): Increased blood–brain barrier permeability in mammalian brain seven days after exposure to the radiation from a GSM-900 mobile phone

L. Hardell, M. Carlberg and K Hansson Mild (Sweden): Epidemiological evidence for an association between use of wireless phones and tumor diseases

M. Kundi and H-P. Hutter (Austria): Mobile phone base stations – effects on wellbeing and health

L.L. Morgan: Estimating the risk of brain tumors from cellphone use: published case–control studies

EMF in the environment

Z. Davanipour and E. Sobel: Long-term exposure to electromagnetic fields and the Risks of Alzheimer's disease and breast cancer: Further biological research

O. Johansson: Disturbance of the immune system by electromagnetic fields: A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment disturbance

A.F. Pourlis: Reproductive and developmental effects of EMF in vertebrate animal models

A. Balmori: Electromagnetic pollution from phone masts: Effects on wildlife

P. Huttunen, O. Hänninen and R.Myllylä: FM-radio and TV tower signals can cause spontaneous hand movements near moving RF reflector

C. Blackman: Cell Phone Radiation: Evidence from ELF and RF studies supporting more inclusive risk identification and assessment

Science as a guide to public policy

D. Gee: Late Lessons from early warnings: Towards realism and precaution with EMF?

C. Sage and D.O. Carpenter: Public Health Implications of Wireless Technologies

Special Issue on EMF

Bioelectromagnetics, the study of biological effects of electromagnetic fields (EMF), is an interdisciplinary science with a technical literature that is not easily accessible to the non-specialist. To increase access of the public to the technical literature and to the health implications of the scientific findings, the Bioinitiative Report was organized by an international group of scientists and published online at www.bioinitiative.org on August 31, 2007. The report has been widely read, and was cited in September 2008 by the European Parliament when it voted overwhelmingly that the current EMF safety standards were obsolete and needed to be reviewed.

This special issue of Pathophysiology includes scientific papers on the EMF issue by contributors to the Bioiniative Report, as well as others, and is prepared for scientists who are not specialists in bioelectromagnetics. Each paper is independent and self-contained. To help the reader appreciate how the different subjects contribute to an understanding of the EMF issue, the papers are arranged in groups that emphasize key areas, and the role of science in analyzing the problem and evaluating possible solutions. The subject headings are:

- DNA to show biological effects at the sub-cellular level that occur at very low EMF thresholds and across frequency ranges of the EM spectrum. Interactions with DNA may account for many of the effects of EMF, and they raise the possibility that genetic damage due to EMF can lead to cancer.
- The Brain is exposed to radiation from mobile phone antennas, and laboratory studies show that the radiation causes leakage of the protective blood–brain barrier, as well as the death of neurons in the brain. Radiation emitted from base stations can affect all who are in the vicinity. Epidemiological studies have shown a relation between exposure to mobile phones, base-stations and the development of brain tumors. Some epidemiological studies have significant flaws in design, and the risk of brain cancer may be greater than reported in the published results.
- In addition to the risk of brain cancer, EMF in the environment may contribute to diseases like Alzheimer's dementia and breast cancer in humans, as well as reproductive and developmental effects in animals in the wild. EMF affect the biochemical pathways and immunological mechanisms that link the different organ systems in our bodies and those of animals. The human body can act as an antenna for RF signals, and a small percentage of the population appears to be so sensitive to EMF that it interferes with their daily lives. In addition to the growing presence of EMF signals in the environment, the complexity of the signals may be important in altering biological responses. These are among the many factors that must be considered in approaching EMF safety issues.
- Science as a guide to public policy

Four centuries ago, when Francis Bacon envisioned a course for modern science, he expressed the idea that *knowledge is power that should be applied for the benefit of mankind*. It is in keeping with that ethical standard that the final papers in this issue show how knowledge gained from scientific research can help solve problems arising from EMF in our environment. The first of these papers discusses the Precautionary Principle, its growing acceptance as a rational approach to environmental issues, and how past experience can help us deal with the EMF issue. The second paper, by the editors of the original BioInitiative Report, is an update on how best to deal with the challenge of EMF in the environment and, specifically, the problems accompanying wireless technologies. The last paper describes the most recent in a

series of petitions by scientists demanding that society use our knowledge to deal effectively with the EMF issue.

We trust that the reviews and original research papers will increase awareness of the growing impact of EMF in the environment, and the need for modern society to deal expeditiously with the potential health problems brought to light by EMF research.

Guest Editor
Martin Blank
Physiology and Cellular Biophysics, Columbia University, New York, USA
E-mail address: mb32@columbia.edu

22 January 2009

ELSEVIER

Pathophysiology 16 (2009) 217–231

ISP
PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Late Lessons from Early Warnings: Towards realism and precaution with EMF?

David Gee *

European Environment Agency, Kongens Nytorv 6, DK-1050 Copenhagen K, Denmark

Received 17 December 2008; accepted 30 January 2009

Abstract

The histories of some well-known public and environmental hazards, from the first scientifically based early warnings about potential harm, to the subsequent precautionary and preventive measures, have been reviewed by the European Environment Agency in their report "Late Lessons from Early Warnings: The Precautionary Principle 1896–2000". This paper summarises some of the definitional and other issues that arise from the report and subsequent debates, such as the contingent nature of knowledge; the definitions of precaution, prevention, risk, uncertainty, and ignorance; the use of different strengths of evidence for different purposes; the nature and main direction of the methodological and cultural biases within the environmental health sciences; the need for transparency in evaluating risks; and public participation in risk analysis. These issues are relevant to the risk assessment of electro-magnetic fields (EMF). Some implications of these issues and of the "late lessons" for the evaluation and reduction of risks from EMF are indicated.

Keywords: Late lessons; EMF; Precautionary principle, Evaluating evidence

1. Introduction

The histories of fourteen well-known hazards and their harm, which include some chemicals: tributyl tin (TBT), benzene, polychlorinated biphenyls (PCBs), chlorinatedfluorocarbons (CFCs), methyl tert butyl ether (MTBE), sulphur dioxide, (SO_2) and Great Lakes pollution; two pharmaceuticals (diethylstilboestrol (DES) and beef hormones); two physical agents (asbestos and medical X-rays); one pathogen (BSE); and fisheries, have been reviewed by the European Environment Agency [1]. The purpose of the review was to see *how societies had used*, or not, the available scientific information in order to avoid or reduce hazards and risks, and at what overall cost.

Twelve "Late Lessons" were drawn which attempted to synthesise the very different experiences from the case studies into generic knowledge that can help inform decision making on potential hazards from, for example, GMOs [2,3], nanotechnologies [4], mobile phones [5,6] and such endocrine disrupting substances as phthalates, atrazine and bisphenol A [7–9]. These emerging issues are all cases for which the luxuries of hindsight are not yet available but where there is some plausible evidence of harm, and where exposures are widespread *and generally rising*.

The purpose of the twelve late lessons is to help societies to make the most of both past experience and current knowledge in order to anticipate and reduce the impact of future "surprises" from technologies, without stifling innovation.

The "late lessons" are reproduced in Box 1 .

2. The early use of precaution

John Graham, who was senior science policy advisor to President Bush, is a critic of the precautionary principle, but has nevertheless noted that:

Precaution, whether or not described as a formal principle, has served mankind well in the past and the history of public health instructs us to keep the spirit of precaution alive and well [10].

* Tel.: +45 33 36 71 42; fax: +45 33 36 71 28.
E-mail address: David.Gee@eea.eu.int.

0928-4680/$ – see front matter © 2009 Published by Elsevier Ireland Ltd.
doi:10.1016/j.pathophys.2009.01.004

Box 1: "The EEA Twelve Late Lessons"

A. "Identify/Clarify the Framing and Assumptions"

1. Manage "uncertainty" and "ignorance" as well as "risk".
2. Identify and reduce "blind spots" in the sciences used.
3. Assess and account for all pros and cons of action/inaction.
4. Analyse and evaluate alternative options to the agent/activity under scrutiny.
5. Take account of stakeholder values.
6. Avoid "paralysis by analysis" by acting to reduce hazards via the precautionary principle.

B. "Broaden Assessment Information"

7. Identify and reduce interdisciplinary obstacles to learning.
8. Identify and reduce institutional obstacles to learning.
9. Use "lay" and local as well as specialist knowledge.
10. Identify and anticipate "real world" conditions.
11. Ensure regulatory and informational independence.
12. Use more long-term (i.e. decades) monitoring and research.

Graham might have been thinking of the cholera episode of 1854 in Soho, when precaution did indeed serve the people of London well. Dr. John Snow, a well known but controversial London physician, was called in to investigate the cholera outbreak. He used the spirit of precaution to advise banning access to the polluted water of the Broad St. pump, which he suspected was the cause of a serious cholera outbreak. He based his recommendation partly on the evidence he had gathered from his comparative study of two South London populations, who were separately served by piped or well water; and partly on his innovative spatial epidemiological study of the Soho area which pointed to the Broad St. well as the source of water polluted by faeces. He considered this overall evidence was sufficiently strong to justify advising the precautionary action of removing the water pump handle, so that consumers would be forced to use less convenient but cleaner water supplies. His view was accepted by the local church authorities who administered the area.

We know now that Snow's conclusion was accurate. However, his views on cholera causation were not shared by the medical establishment of the day, the Royal College of Physicians and the London Board of Health, who had considered Snow's thesis and rejected it as 'untenable' and biologically implausible [1]. They believed that cholera was caused by airborne, not water borne, pollution. Their scientific "certainty" was increasingly challenged by Snow and others until Koch in Germany finally isolated the cholera vibrio in 1883, thus removing the last remaining doubt about the veracity of Snow's water pollution hypothesis.

The Snow story illustrates many of the key elements of the PP issue that are relevant to today's health and environment controversies, viz conflicting expert advice; competing scientific paradigms; the strength of scientific evidence needed to justify action; the long time lag between *observing* compelling associations and *understanding* their mechanisms of action; and the pros and cons of being wrong in taking action to remove risks, compared to the pros and cons of inaction.

The histories of TBT, PCBs and the other cases in the EEA "Late Lessons" report provide further illustrations of these points.

3. On paradigms and mechanisms of action

Scientists can cling to their favourite paradigm for decades—as with supporters of the air pollution theory in the cholera example between 1854 and 1883, despite mounting evidence that they are likely to be wrong. This passion for the prevailing paradigm is not uncommon. Max Planck, the Nobel physicist noted darkly that old paradigms only really die out when their promoting professors also die: "A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it" [11].

In similar vein, the IPPC has cautioned the scientific authors of its climate change assessment reports against:

a tendency for a group to converge on an expressed view and become over confident in it. Views and estimates can also become anchored on previous versions or values to a greater extent than is justified [12].

This "power of the prevailing paradigm" is relevant to the current controversy over mobile phones, where the dominant view of WHO, the EU, and many others is that EMF-RF (radio frequency) energy has to be sufficiently large to cause the heating of biological tissue if it is to cause significant harm [13–15]. The current ICNIRP guidelines for limiting unacceptable RF exposures are derived from this paradigm and are therefore:

based on short term, immediate health effects, such as stimulation of peripheral nerves . . . and elevated tissue temperatures [13].

This majority view is opposed by those who think that much lower levels of EMF have the potential to cause harm via their capacity to disturb cell signalling or stress response systems that use very small changes in electro-magnetic fields [16–19].

Is the EMF field witnessing one of those shifts in prevailing paradigms that Thomas Kuhn noted had characterised progress in many fields of science? [20]

It can be difficult to accept that something is happening if you do not understand how it can be happening. A major reason why some scientists hang on to their preferred paradigm when evidence against it is mounting is that they need not only to observe a strong association between a cause and an effect but also to understand the mechanisms of biological action that link them. However, this can take decades. From the *association* between exposure to water polluted with human faeces and cholera, observed by Snow in 1854, to Koch's discovery of the *mechanism of action*, took 30 years of further scientific inquiry.

Such a long time lag between acknowledging compelling associations and understanding their mechanisms of action is a common feature of scientific inquiry, as illustrated by many of the case studies in the EEA report. Biological and ecological understanding about exactly how these exposures caused harm is still absent, decades after the associations were accepted as sufficient to justify preventive actions.

With EMF, there is currently no *established* knowledge about the mechanisms of biological action that could explain the consistent associations between EMF-ELF (extremely low frequency) exposure from overhead electrical power lines and childhood leukaemia. However, there is some evidence of *plausible* biological mechanisms. These include hypotheses concerning "information physics" [21]; melatonin [22]; oxidative stress [19]; indirect effects via cancer promotion; and the radical pair mechanism, which according to the Swedish Radiation Protection Authority, is "*probably the most plausible hypothesised mechanism*" [23]. Some or all of the above mechanisms, possibly in combination with other stressors and genetic configurations, is likely to eventually provide mechanistic explanations for the observed biological effects of EMF-ELF.

Despite this lack of mechanistic knowledge, and a general lack of corroborating animal evidence, the International Agency for Research on Cancer (IARC-WHO) recognised ELF from such magnetic fields as possibly carcinogenic in 2002, based on more than 30 positive epidemiological studies which had been completed since the first "early warning" observation in 1979 [24]. Other scientists do not believe the association between ELF and childhood leukaemias, given the paucity of mechanistic knowledge. However, recent animal and human evidence seems to be filling some of this knowledge gap [25].

The ELF story has parallels with that concerning the ionising X-rays which were routinely given to pregnant women before the early warning of Alice Stewart in the 1950s. She had observed a twofold excess of childhood leukaemias in women given X-rays during pregnancy. Her findings were eventually accepted by the 1970s, despite the continuing absence of knowledge about mechanisms of action: and such routine X-ray exposures were then stopped [26].

The current situation with the EMF-RF exposures from mobile phones is characterised by some positive yet generally inconsistent epidemiological evidence [27–29], by a general absence of animal evidence; and by little established knowledge of possible mechanisms of carcinogenic action.

The question therefore arises: should actions that seem likely to protect the health of the public have to wait for knowledge about mechanisms of action? The precautionary principle was designed to justify actions to protect the public and the environment in the absence of some significant knowledge, and could be used to justify exposure reductions to EMF, despite current gaps in knowledge.

Could the unfolding story of EMF be a repetition of these earlier histories of ionising radiation exposures where evidence of harm was only "established" some twenty or more years after the first early warning?

4. Early warnings

When dealing with newly emerging hazards it can be helpful to use historical examples to illustrate what a scientifically based early warning looks like. It is often difficult to properly recognise such warnings when they occur.

A good example is that provided by the UK Medical Research Council's Swann Committee in 1969. The Committee was asked to assess the evidence for risks of resistance to antibiotics in humans, following the prolonged ingestion of trace amounts of antibiotics arising from their use as growth promoters in animal feed [30]. They concluded that:

Despite the gaps in our knowledge ... we believe ... on the basis of evidence presented to us, that this assessment is a sufficiently sound basis for action ... The cry for more research should not be allowed to hold up our recommendations'. ...'sales/use of AFA should be strictly controlled via tight criteria, despite not knowing mechanisms of action, nor foreseeing all effects [31].

Despite the gaps in knowledge, the need for much more research, and considerable ignorance about the mechanisms of action, the available evidence was acknowledged by the Swann Committee as sufficient to justify the need for the authorities to restrict the possibility of public dietary exposures to antibiotics from animal growth promoters.

This early warning was initially heeded, but was then progressively ignored by the pharmaceutical companies and regulatory authorities, which wanted more scientific justification for restricting profitable anti-microbial growth promoters. However, the use of antibiotics as growth promoters was finally banned in the EU in 1999, following the lead of Sweden in 1985 [30].

Pfizer, the main supplier of such antibiotics in Europe, appealed against the European Commission decision to ban their product, pleading, inter alia, an insufficiency of scientific evidence. They lost the case at the European Court of Justice [32]. This case further clarified the appropriate use and

application of the precautionary principle in circumstances of scientific uncertainty and of widespread, if low, public exposures to a potentially very serious threat.

On EMF there has been a number of early warnings about potential risks at low levels of exposure, culminating in the Bioinitive report of 2007 [33]. This prompted the EEA to also issue an "early warning":

Appropriate, precautionary and proportionate actions taken now to avoid plausible and potentially serious threats to health from EMF are likely to be seen as prudent and wise from future perspectives [34].

It is possible that such early warnings, particularly on RF from mobile phones, issued by the EEA and others, will turn out to be incorrect. This will only be established with time, and the hindsight it brings. However, the EEA would rather be wrong in raising concerns that turn out not to be justified, than being wrong in not issuing an early warning if the potentially serious hazards from RF technology turn out to be real. Large numbers of people are potentially exposed to RF, particularly children who are generally more susceptible to the potential harm. Reducing RF exposures in response to a mistaken early warning is preferable to not reducing exposures to a hazard that turns out to be real, and largely irreversible. Moreover, encouraging such reduction could help to stimulate technical innovation.

5. The importance of timing

The issue of time is a critical issue for risk analysis and application of the precautionary principle.

For example, the time from the first scientifically based early warnings (1896 for medical X-rays, 1897 for benzene, 1898 for asbestos), to the time of policy action that effectively reduced damage, was often 30–100 years, during which exposure increased considerably (Table 1).

One consequence of such failures to act in good time (e.g. on CFCs or asbestos) is greater and irreversible damage over longer time periods. For example, extra natural radiation coming through the ozone hole will cause many tens of thousands of extra skin cancers in today's children but the cancers will only peak around the middle of this century because of the long latent period between exposure and effect. Over a decade's worth of extra skin cancers could have been avoided if action had been taken on the first early warning, (which was subsequently deemed robust enough to justify giving the Nobel prize for Chemistry to its authors), rather than on the discovery of the ozone hole itself. Other negative impacts from the damaged ozone hole include eye cataracts and reduced crop productivity.

Such long-term but foreseeable impacts raise liability and compensation issues, including appropriate discount rates (if any) on future costs and benefits. These issues, which involve value and equity choices, need also to be discussed by stake-

Table 1

Late Lessons chapter	Date of first Early Warning	Date of Effective risk reduction action	Years of substantial inaction
Fisheries: taking Stock	1376	1995–2008 "responsible" management: which is not very effective	Hundreds...
Radiation: Early Warnings, Late Effects	1896	1961–1996 UK etc., then EU laws	65
Benzene: occupational setting	1897	1978 Benzene voluntarily withdrawn from most consumer products, US	81
Asbestos: from "magic" to malevolent material	1898	1999 EU ban by 2005	101
PCBs and the Precautionary Principle	1899	1970–80s:EU and US restrictions; phase out by 2010	c. 100
Halocarbons, the ozone layer and the Precautionary Principle	1974	1887–2910 global ban on CFCs + other Ozone depleters	10–30
DES: long-term consequences of pre-natal exposure	1938	1971–1985 US, EU, global ban	30–50
Antimicrobials as growth promoters: resistance to common sense	1969	1999 EU ban	30
SO_2: from protection of human lungs to remote lake restoration	1952 (lung) 1968 (lakes)	1979–2001 increasing EU etc restrictions leading to c 90% reduction on 1975 levels by 2010	25–55
MTBE in petrol as a substitute for lead	1960 taste/odour/persistence in water	2000 undesireable in Denmark/California: permitted elsewhere	40+
Great Lakes contamination	1962/3	1970s DDT banned in N America& EU. 2000 debates continue about persistent health damaging pollution	10–?
TBT antifoulants: a tale of ships, snails and imposex	1976–81 French oysters collapse	1982–7 French, UK then NE Atlantic ban; 2008 global ban	5–30
Beef Hormones as growth promotors	1972/3 oestrogen effects on wildlife	1988 EU ban, US continues	16+
Mad cow disease-reassurances undermined precaution	1979–1986	1989 Partial; 1996 total ban	10–17

holder groups. Experience in the climate change field with these long-term issues [35] may be helpful for the EMF issue.

Timing is also a critical issue for the assessment of risks. Many agents seem to be most damaging during sensitive windows of biological opportunity, either at the foetal stage of development [36], or when the host is susceptible because of an immune response deficiency, or of impacts from other stressors.

Timing is relevant to several biological end points as indicated in a review of the evidence on endocrine disrupting substances:

the time of life when exposures take place may be critical in defining dose–response relationships of Endocrine disrupting substances for breast cancer as well as for other health effects [37].

Responding to these issues of timing involves using lower strengths of evidence to justify action at earlier times in the exposure history of the stressors that inflict damage during specific windows of vulnerability, such as during foetal or early childhood development [38]. The wide exposure of children to EMF brings the timing of actions to reduce exposures into critical focus.

6. Knowledge and ignorance, prevention and precaution

The Broad St. pump example, and the other case studies in the EEA report serve to illustrate the contingent nature of scientific knowledge. Today's scientific certainties can be tomorrow's mistakes, and today's research can both reduce and increase scientific uncertainties, as the boundaries of the "known" and the unknown expand (Fig. 1).

It is common to hear the call for "more research" to remove uncertainties before any actions are taken to reduce hazards. However, such further research may not only take many years but tomorrow's knowledge, in addition to removing some uncertainties, is likely to identify previously unknown

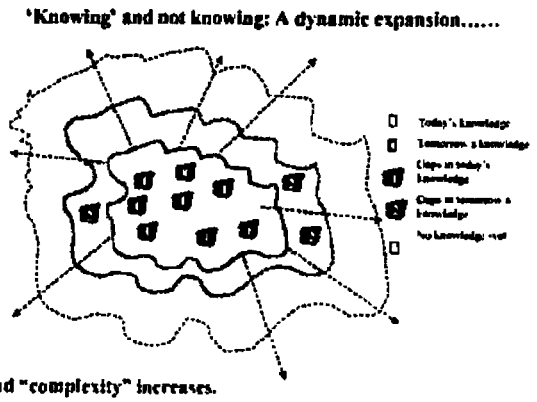

Fig. 1. Knowing and not knowing both espand.

sources of both uncertainty and ignorance. These new uncertainties can then be used as reasons for continued inaction on hazard reduction: "paralysis by analysis".

Socrates observed some time ago:

I am the wisest man alive, for I know one thing, and that is that I know nothing [39].

Such an approach to knowledge encourages humility in scientists rather than the hubris demonstrated by those scientists who, for too many years, professed certainties about the absence of harm from X-rays, asbestos, CFCs etc. These "certainties" turned out to be misplaced as knowledge expanded [1].

Many great scientists since Socrates have also displayed much humility in the face of acknowledged ignorance. Isaac Newton provided an elegant illustration of this towards the end of his life of discoveries:

to myself I seem to have been only like a boy playing on the seashore, and diverting myself now and then, finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me [40].

This was an early lesson in humility that seems to have been lately forgotten by many of the scientists and politicians who deal with hazards to the public and environment.

The distinction between uncertainty and ignorance also has significant implications for risk analysis and management [41]. Uncertainties arise, inter alia, from the known gaps in knowledge, from imprecise exposure sampling and monitoring; and from the assumptions and simplifications of models used to describe complex reality. Scientists involved in regulatory risk assessments try to take account of some of these uncertainties by using arbitrary safety factors to arrive at "acceptable" exposure limits.

Acknowledging ignorance, however, involves acknowledging the unknown unknowns, as well as the sometimes unknowable unknowns that arise from complex and unpredictable biological and ecological systems and the random variations that are common to them [42,43]. It is obviously not possible to just use safety factors applied to "known" associations to account for such lack of knowledge.

States of ignorance are also the source of new scientific discoveries as well as of unpleasant "surprises" such as the mesothelioma cancer from asbestos, the hole in the ozone layer, or the reversed sexuality in the sea snails contaminated by the TBT biocide in marine anti-fouling paints [44].

Foreseeing and preventing hazards in the context of ignorance presents particular challenges to decision-makers. Ignorance ensures that there will always be surprises, and at first sight it looks impossible to do anything to avoid, or mitigate, them. However, there are some measures that could help minimise the consequences of ignorance and the impacts of surprises:

- using the intrinsic properties of potential stressors as generic predictors for unknown but possible impacts e.g.

the persistence, bioaccumulation and spatial range potential of chemical substances [45];

- reducing specific exposures to potentially harmful agents on the basis of credible 'early warnings' of initial harmful impacts, thus limiting the size of any other 'surprise' impacts from the same agent, such as the asbestos cancers that followed asbestosis; and the PCB neurotoxicological effects that followed its wildlife impacts;
- promoting a diversity of robust and adaptable technological and social options to meet human needs, which then limits technological 'monopolies' (such as those of asbestos, CFCs, PCBs etc.), and therefore reduces the scale of any 'surprise' from any one technological option;
- accepting significant biological and ecological effects, such as inflammatory responses, or changing sex ratios, as sufficient evidence of potentially adverse effects to justify hazard reduction, without waiting for the adverse effects themselves to arrive;
- using more long-term research and monitoring of what appear to be "surprise sensitive sentinels", such as frogs, bees and foetuses, in order to identify "early warnings" earlier;
- using scenarios and stakeholder involvement to help foresee and anticipate implications of particular technological and social pathways.

Some of these approaches are relevant to EMF.

The distinction between *prevention* and *precaution* is also important. Preventing hazards from "known" risks is relatively easy and does not require precaution. Banning smoking, or asbestos, today requires only acts of *prevention* to avoid the well-known risks. However, it would have needed *precaution* (or foresight, based on a lower strength of evidence), to have justified exposure reductions to the then uncertain hazards of asbestos exposure in the 1930s–50s, or of tobacco smoke in the 1950s–60s.

Such precautionary acts then, if implemented successfully, would have saved many thousands of lives and, in the case of asbestos, stimulated innovation in the insulation and other asbestos using industries decades earlier than has been the case.

Similarly, it would need precaution to justify reducing exposures to an IARC category two carcinogen, such as EMF, but only prevention to avoid the cancer risk from a class one carcinogen, such as ionising radiations, where the evidence for action is very well established.

There has been much debate generated by the different meanings attached to these and other terms commonly used in debates on hazards, such as "prevention", "precaution", "risk", "uncertainty" and "ignorance". Table 2 attempts to clarify these definitions, using some of the "Late Lessons" case studies as illustrations.

There is also frequent confusion between the *strength of evidence* needed to justify any action to reduce risks, and the *type of action* deemed to be appropriate: the two are not directly connected. For example, there is very strong evidence that cars harm people, but they are not banned from most places. In contrast, slight evidence of possible birth defects arising from taking a pregnancy pill would usually be sufficient to justify banning that pill.

7. The precautionary principle: some definitions and interpretations

The Vorsorgeprinzip, (the "precautionary", or "foresight") principle, only emerged as a specific policy tool during the German debates on the possible role of air pollution as a cause of "forest death" in the 1970–80s.

An increasing awareness of ecological complexity and uncertainty during the 1980–90s led to debates on the Vorsorgeprinzip shifting from Germany to the international level, initially in the field of nature conservation [46] but then particularly in marine pollution, where an overload of data accompanied an insufficiency of knowledge [47]. This absence of knowledge generated the need to act with precaution to reduce the large amounts of chemical pollution entering the North Sea.

Since then over 60 international treaties, including the Third North Sea Ministerial Conference, 1990, have included reference to the precautionary principle, or, as the Bush negotiators prefer to say, the precautionary approach. (A recent legal review points out that there is little, if any practical difference between these two concepts [48].)

The Treaty of the European Union cites the precautionary principle thus:

Community policy on the environment ... shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should, as a priority, be rectified at the source, and the polluter should pay [49].

Although only cited in the environment part of the EU Treaty, the precautionary, prevention and polluter pays principles also apply to health and consumer affairs, as European Court of Justice decisions have made clear [50].

Unfortunately, these principles, as well as the important and legally required *proportionality principle*, which limits disproportion between the costs and benefits of precaution or prevention, are not defined in the EU Treaty. However, their usage has been clarified in over 100 court cases [48].

A definition of the precautionary principle that is often cited by supporters and detractors alike is that from the The North Sea Declaration, which calls for:

action to avoid potentially damaging impacts of substances, even where there is no scientific evidence to prove a causal link between emissions and effects (my emphasis).

Critics of the precautionary principle claim that this definition appears to justify action even when there is "*no scientific evidence*" that associates exposures with effects. However, the N. Sea Conference text clearly links the words "no scien-

Table 2
Towards a clarification of key terms.

Situation	State and dates of knowledge	Justification for action
• Risk	Known' impacts; 'known' probabilities e.g. asbestos 1999	*Prevention*: action taken to reduce known hazards e.g. eliminate exposure to asbestos dust
• Uncertainty	'Known' impacts; 'unknown' probabilities e.g. antibiotics in animal feed and associated human resistance to those antibiotics 1999	*Precautionary Prevention*: action taken to reduce exposure to plausible hazards e.g. ban antibiotic growth promotors
• Ignorance	'Unknown' impacts and therefore 'unknown' probabilities e.g. the 'surprise' ozone hole from (CFCs), pre-1974	*Precaution*: action taken to anticipate, identify and reduce the impact of 'surprises'

Source: Amended from the "Late Lessons" report, EEA 2001.

tific evidence" with the words "to prove a causal link" (my emphasis).

We have already seen with the Broad St. pump example that there is a significant difference between the evidence needed to show an "*association*" between a pollutant and its harm, and evidence which is robust enough to "prove" a *causal* link, which requires a very much higher strength of evidence. Bradford Hill pointed this out in his classic paper on association and causation in public health which he wrote at the height of the smoking controversy [51].

The N. Sea Declaration says that the absence of the strong evidence needed to support causality is not a valid reason for inaction where there is widespread and potentially hazardous exposures and some plausible evidence of potential harm.

Despite increasing use of the precaution principle there is still much disagreement and discussion about its practical application. This is particularly due to the absence of an EU definition in regulatory texts, and to disputes over the sufficiency of scientific evidence needed to justify public policy action.

For example, many "definitions" of the precautionary principle or approach in the 60 or so Treaties and Conventions that now include this concept use a triple negative: that is, they identify the *absence* of strong scientific evidence (e.g. of "full" certainty") as a reason that *cannot* be used to justify *not* acting, And they do not specify what a sufficiency of evidence would be that could justify taking action.

Some other widely cited definitions of the precautionary principle, notably the Wingspread and UNESCO definitions, are rather long, and include items that are not strictly part of a definition, such as the *process* by which decisions are taken (i.e. participatory, or not); and the allocation of the *burden of proof* to risk makers or risk takers: the latter is a separate issue that societies have dealt with without recourse to the precautionary principle.

For example, European and other societies have long placed the pre-market burden of establishing reasonable grounds for the safety of medicines, pesticides, nuclear plants and large construction projects on those who wish to provide such products or projects, Other potentially harmful agents, such as the 100,000 or so existing chemicals in consumer products, have been placed on the market without such pre-market burdens. Although pre-market testing or assessment is more precautionary than post market surveillance, it does not require justification from the precautionary principle.

There have been further definitions and clarifications of the precautionary principle from, for example from the EU Council of Ministers; in EU case law; and in the regulation establishing the new European Food Safety Authority, EFSA [52].

The judgement of the European Court of Justice in the BSE case illustrated a general definition which many authoritative commentators consider contains most of the necessary elements of the precautionary principle:

Where there is uncertainty as to the existence or extent of risks to human health, the institutions may take protective measures without having to wait until the reality and seriousness of those risks become fully apparent [53].

The WHO Declaration from the Fourth Ministerial Conference on Environment and Health [54] also refers to the precautionary principle. An explanatory background paper recommends that the principle:

should be applied where the possibility of serious or irreversible damage to health or the environment has been identified and where scientific evaluation, based on available data, proves inconclusive for assessing the existence of risk and its level but is deemed to be sufficient to warrant passing from inactivity to policy alternatives [55].

A recent report from the Health Council of the Netherlands on the precautionary principle provides a clear and cogent summary of the issues raised by its use [56].

However, there remains an absence of a clear definition at EU level so the European Environment Agency (EEA), in response to the debates on the precautionary principle since its 2001 report, has produced a working definition of the precautionary principle.

The Precautionary Principle provides justification for public policy actions in situations of scientific complexity, uncertainty and ignorance, where there may be a need to act in order to avoid, or reduce, potentially serious or irreversible threats to health or the environment, using an appropriate level of scientific evidence, and taking into account the pros and cons of action and inaction [8].

The definition is proving useful in promoting a shared understanding of the precautionary principle. It is explicit in specifying both uncertainty and ignorance as contexts for applying the principle; it is couched in the affirmative rather

than the negative; and it explicitly acknowledges that a case specific sufficiency of scientific evidence is needed to justify public policy actions, given the pros and cons of action or inaction.

The definition also explicitly widens the conventionally narrow, and usually quantifiable, interpretation of costs and benefits to embrace the wider and sometimes unquantifiable, "pros and cons". Some of these wider issues, such as loss of public trust in science, are unquantifiable, but they can sometimes be more damaging to society than the quantifiable impacts: they therefore need to be included in any comprehensive risk assessment.

But what is "an appropriate strength of evidence" that would justify taking action under the precautionary principle to reduce exposures and risks?

8. Establishing evidence for action

All serious applications of the precautionary principle require some plausible evidence of an association between exposures and current, or potential, impacts.

For example, the Communication from the EU on the precautionary principle [57] specifies that "reasonable grounds for concern" are needed to justify action, but it does not say that these grounds will vary with the specifics of each case: nor does it explicitly distinguish between risk, uncertainty and ignorance.

The strength of scientific evidence that would be appropriate to justify public action clearly must vary with the pros and cons of being wrong with action or inaction in the specific circumstances of each case. These circumstances include the nature and distribution of potential harm; the justification for, and the benefits of the agent or activity under suspicion; the availability of feasible alternatives; and the overall goals of public policy. Such policy goals can include the achievement of the "high levels of protection" of public health, of consumer safety, and of the environment, required by the EU Treaty.

The use of different strengths of evidence for different purposes is not a new idea.

For example, a high strength of evidence such as "beyond all reasonable doubt" is used to achieve good science where A is generally accepted as causing B only when the evidence is very strong. Such a high level of proof is also used to minimise the costs of being wrong in the criminal trial of a suspected murderer, where it is usually regarded as better to let several guilty men go free, when reasonable doubt about their guilt cannot be eliminated, than it is to wrongly convict an innocent man.

However, in a different trial setting, where a citizen seeks compensation for harm that is possibly due to negligent treatment at work, the courts in many European and other societies will use a lower strength of evidence, commensurate with the costs of being wrong in this different situation. An already injured party is given the benefit of the doubt by the use of a medium level of proof, such as "balance of evidence, or probability". This is justified on the grounds that it is more acceptable to give compensation to someone who was *not* treated negligently than it is to *not* provide compensation to someone who was treated negligently. The "broad shoulders" of insurance companies are seen as able to bear the costs of mistaken judgements rather better than the much narrower shoulders of an injured citizen.

In each of these two illustrations it is the nature and distribution of the costs of being wrong that determines the strength of evidence that is "appropriate" to the particular case, based essentially on ethical grounds. The choice of an appropriate strength of evidence in each case is therefore a societal not a scientific issue.

This has long been recognised. Bradford Hill, cited above, drew attention to the social responsibility of scientists whose work involves public health. He concluded his classic 1965 paper on association and causation in environmental health with a "call for action" in which he also proposed case specific and differential strengths of evidence.

His three illustrative examples ranged from "relatively slight" to "very strong" evidence, depending on the nature of the potential impacts and of the pros and cons of being wrong. These varied between a possibly teratogenic medicine for pregnant women; a probable carcinogen in the workplace; and government restrictions on public smoking or diets [51].

In the field of cancer, the International Agency for Research on Cancer also uses several types of scientific evidence to categorise their strengths of evidence on carcinogens [58].

Identifying an appropriate strength of evidence has also been an important issue in the climate change debates. The International Panel on Climate Change (IPCC) discussed this issue at length before formulating their 1995 conclusion that "on the balance of evidence" mankind is disturbing the global climate. They further elaborated on this issue in their 2001 report where they identified seven strengths of evidence that can be used to characterise the scientific evidence for a particular climate change hypothesis. By 2007 the evidence for human induced climate change had strengthened to a "reasonable certainty" [59].

Table 3 provides the middle 5 of these strengths of evidence from the IPPC and illustrates their practical application to a variety of different societal purposes.

In the risk assessments of EMF published so far there has been little explicit discussion about the choice of the strength of evidence used in the assessments. The vague term "no established evidence" is often used to characterise the absence of some strength of evidence that would convince the particular scientists doing the risk assessment that a hazard existed. There is little if any discussion about for whom the evidence is said to be not established (risk takers or risk makers), nor about for what purpose (warning labels, or low cost exposure reductions, for example.).

Table 3
Different levels of proof for different purposes.

Different levels of proof for different purposes: some examples and illustrations			
Probability	Quantitative descriptor (Probability bands based on IPCC 2001)	Qualitative descriptor	Illustrations
100% probability	Very likely 90–99%	• "Statistical significance"	• Part of strong scientific evidence for "causation"
		• "Beyond all reasonable doubt"	• Most criminal law. And the Swedish Chemical law, 1973, for evidence of "safety" of substances under suspicion-burden of p roof on manufacturers
	Likely (66–90%)	• "Reasonable certainty" • "Sufficient scientific evidence"	• Food Quality Protection Act, 1996 (US) • To justify a trade restriction designed to protect human, animal or plant health under World Trade Organisation Sanitary and Phytosanitary (SPS) Agreement, Art. 2.2, 1995
	Medium Likelihood (33–66%)	• "Balance of evidence"	• Intergovernmental Panel on Climate Change 1995 & 2001
		• "Balance of probabilities"	• Much Civil and some administrative law
		• "Reasonable grounds for concern"	• European Commission Communication on the Precautionary Principle 2000
		• "Strong possibility"	• British Nuclear Fuels occupational radiation compensation scheme, 1984 (20–50% probabilities triggering different awards up to 50% +; which then triggers full compensation)
	Low Likelihood (10–33%)	• "Scientific suspicion of risk"	• Swedish Chemical law, 1973, for sufficient evidence to take precautionary action on potential harm from substances-burden of proof on regulators
		• "Available pertinent in formation"	• To justify a provisional trade restriction under WTO SPS Agreement, Art. 5.7 where "scientific information is insufficient"
	Very Unlikely (1–10%)	• Low risk • "Negligible and in significant"	• Household fire insurance • Food Quality Protection Act, 1996 (US)

Source: EEA (2002).

An exception is the Californian EMF-ELF risk assessment which was much more transparent and explicit about these critical issues [60].

Establishing a sufficiency of evidence for whom, and for what purpose, involves value judgements: such issues therefore require public participation.

9. Public participation in risk analysis

Choosing an appropriate strength of evidence for a particular case is not a scientific issue but a social choice. It is therefore necessary to involve the public in decisions about serious hazards and their avoidance: and to do so for all stages of the risk analysis process, as recommended by several authorative bodies during the last 10 years [61,62,63,64,56,65]. Three of the "twelve late lessons" of the EEA report (numbers 5, 9 and 10 in Box 1) also encourage the involvement of stakeholders at all stages of risk analysis.

Fig. 2 based on the above reports, illustrates the iterative nature of risk assessment, risk management, and risk communication; the links between them; and the involvement of stakeholders at every stage, albeit with different intensities.

The existing International and European arrangements for risk analysis, and for the setting of public exposure limits for EMF and other issues such as food [66], do not seem to reflect these recommendations for opening up the process of risk analysis, including risk assessment, to stakeholder participation. Instead they largely retain the older, linear approach where risk assessment is separated from risk management and communication and where communication is largely one way, i.e., from scientists to managers to the public.

The best available science is therefore a necessary but not a sufficient condition for sound public policy making on potential threats to health and the environment, such as from EMF. Where there is scientific uncertainty and ignorance "it is primarily the task of the risk managers to provide risk assessors with guidance on the science policy to apply in their risk assessments" [67]. The content of this science policy advice, as well as the nature and scope of the questions to be addressed by the risk assessors, need to be formulated by the

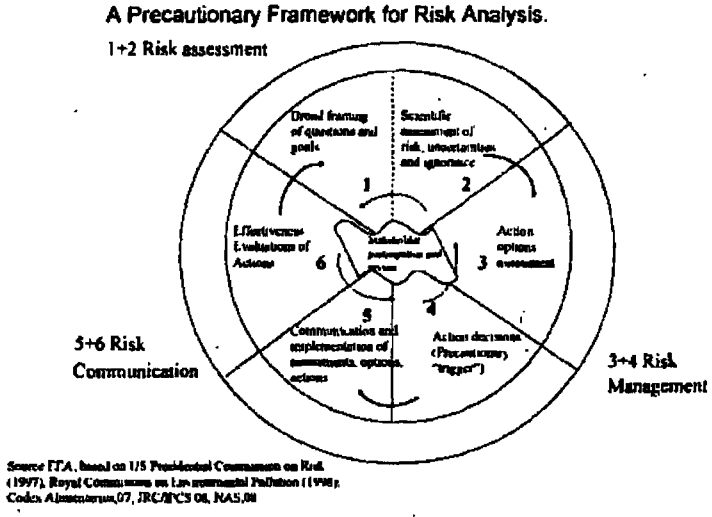

Fig. 2. A Precautionary risk analysis framework.

risk managers and relevant stakeholders at the initial stages of the risk analysis, as indicated in Fig. 2.

It is not easy to involve the public in all stages of risk analysis and in helping to set associated research agendas and technological trajectories [68,69]. However, there are some useful experiences, in both Europe and the USA, with focus groups, deliberative polling, citizens juries, and extended peer review, which are exploring appropriate ways forward [70,71].

The SAGE stakeholder process in the UK, which focused on ELF from power lines, provides a useful illustration of stakeholder engagement [72].

Public participation is particularly essential when future technological and social pathways, and associated hazards, are unpredictable: being wrong together is more socially robust than letting experts alone make the mistakes.

But why are there enough "mistakes", from delayed policy actions to prevent serious harm, to fill several volumes of Late Lessons reports?

10. False positives and false negatives

The fourteen case studies in the Late Lessons Report are all examples of "false negatives" in the sense that the agents or activities were regarded as not harmful for many years before evidence showed that they were harmful. Attempts were made to include a "false positive" case study in the report (i.e. where actions to reduce potential hazards turned out to be unnecessary), but neither authors nor sufficiently robust examples were found.

Providing evidence of "false positives" is more difficult than with "false negatives" [73]. For example, how robust, and over what periods of time, does the evidence on the absence of harm have to be before concluding, with confidence, that a restricted substance or activity is without significant risk?

Volumes 2 of "Late Lessons", which the EEA will publish in 2009, will explore the issues raised by false positives, including lessons to be learned from such apparent false positives as the EU ban on food irradiation and the hazardous labelling of saccharin in the US [74].

But why are there so many "false negatives" that have been so damaging to health or environment? And how might this be relevant to EMF?

The first Late Lessons volume of case studies provided two main answers: the bias within the health and environmental sciences towards avoiding "false positives", which thereby generates more "false negatives": and the dominance within societal decision-making of short term, specific, economic and political interests over the longer term, diffuse, and overall welfare interests of society. The latter point needs to be further explored, particularly by the political sciences: the current and increasing dominance of the short term in markets and in parliamentary democracies makes this an urgent issue.

Since the publication of "Late Lessons" the EEA has further explored the second cause of "false negatives" i.e. the issue of bias within the health and environmental sciences. Table 4 lists eighteen common features of methods and culture in the environmental and health sciences and shows their main directions of error. Most tend towards generating "false negatives".

Table 3 is derived from papers presented to a conference on the precautionary principle organised by the Collegium Ramazzini, the EEA, the WHO and NIEHS in 2002 [75]. It tries to communicate the main directions of the biases within the environmental and health sciences which decision makers and the public should be aware of as they debate the evidence on emerging hazards such as EMF.

Table 4
ON BEING WRONG: Environmental and health sciences and their main directions of error.

Scientific studies	Some methodological features	Main[a] directions of error-increases chances of detecting a:
Experimental	• High doses	• False *positive* (negative for low dose effects)
Studies	• Short (in biological terms) range of doses	• False negative
(Animal Laboratory)	• Low genetic variability	• False negative
	• Few exposures to mixtures	• False negative
	• Few Foetal-lifetime exposures	• False negative
	• High fertility strains	• False negative (developmental/reproductive endpoints)
Observational	• Confounders	• *False positive* (negative with multi-causality?)
	• Recall bias	• *False positive*
Studies	Inappropriate controls	• *False positive*/negative
(Wildlife & Humans)	• Non-differential exposure misclassification	• False negative
	• Inadequate follow-up	• False negative
	• Lost cases	• False negative
	• Simple models that do not reflect complexity	• False negative
Both	• Publication bias towards positives	• *False positive*
Experimental and observational studies	• Scientific cultural pressure to avoid false positives	• False negative
	• Low statistical power (e.g. From small studies)	• False negative
	• Use of 5% probability level to minimise chances of false positives	• False negative
	• Much scrutiny of positive studies cf. negative studies	• False negative

[a] Some features can go either way (e.g. inappropriate controls) but most of the features mainly err in the direction shown in the table.

11. Towards realism about complex reality

Max Planck observed that "reality is ... just a very thin slice of that vast range of what our thoughts try to encompass" [76]. EMF scientists and risk assessors need not only to take account of the false negative/positive biases described above but they should also take more account of "that vast range" of other realities which characterise the EMF issue. These include multi-causality; thresholds; timing of dose; sensitive sub-populations; sex, age, genetics, and immune status of the host; cumulative exposures to EMF and other stressors; information physics; effects below the thresholds of such "acute" impact as tissue heating; non-linear dose–response relationships; "low dose" effects; the absence of unexposed controls; and the effects arising from disturbing the balance between opposing elements in complex biological systems, i.e. the "harmony of opposites" which Heraclitus noted many centuries ago.

In the EMF debate these complexities are often subsumed under many simplifying assumptions. For example, the WHO review of power line ELF states that:

Based on known physical principles and a simplistic biological model, many authors have argued that average magnetic fields of 0.3–0.4 micro tesla are orders of magnitude below levels that could interact with cells or tissues and that such interactions are thus biophysically implausible [77].

In the context of expanding scientific knowledge, the "implausibility" of biological interactions may not be a robust basis on which to dismiss positive epidemiological or experimental observations, especially when the biological models being used are "simplistic".

The case studies in the EEA report illustrate the surprises that arise from real life ecological and biological complexities and which may carry some lessons for the EMF debate. For example, the unfolding of the TBT story was accompanied by an increased appreciation of scientific complexity. This arose from the discoveries that the known acute effects provided no indication of the chronic impacts that were caused by very low doses (i.e. in parts/trillion); that high exposure concentrations were found in unexpected places e.g. in the marine micro-layer; and that bioaccumulation in higher marine animals, including sea-food for human consumption, was much greater than expected. The early and prescient actions on TBT exposure reduction in France and the UK in 1982–85 were based only on a medium 'strength of evidence' for the 'association': evidence that was sufficient to infer 'causality', or to identify 'mechanisms of action' came much later.

We were lucky with TBT: a highly specific, initially uncommon impact (imposex) was quickly linked to one chemical, TBT. This is not likely to happen with the multi-causal and more common impacts such as neurodevelopmental diseases and dysfunctions, or cancers, which are the more complex impacts from EMF that are under suspicion.

Some key lessons from the DES story are also relevant to EMF exposures [78].

These include the realisation that the absence of visible and immediate teratogenic effects is not robust evidence for the absence of reproductive toxicity; and the timing of the dose clearly determined the poison, in contrast to the conventional dictum in toxicology, articulated by Paracelsus, that 'the dose determines the poison'.

DES is now a well-studied compound, with over 20,000 publications, yet many doubts persist about its mechanisms of action more than 30 years after it was banned on compekking observatory evidence that has since become more so. If we still have few biological certainties about DES after so much time and research, what should our attitude be towards relatively little understood hazards, such as other endocrine disrupting substances and EMF?

The scientists and risk assessors of EMF need not only to acknowledge the "surprises" that arise from complex realities but also the asymmetry of measurement precision between gene typing and environmental exposure assessment. As Vineis has observed, such asymmetry is likely to lead to an underestimation of the effects of environment and an overestimation of the effects of genes in the gene/environment interactions that are involved in most public health issues, including EMF [79].

The research implications arising from multi-causality, and from the systemic interactions between genes, host conditions and environmental stressors, seem not to have been fully recognised in the environmental and health sciences.

Sing has noted that:

neither genes nor their environments, but their interactions, are causations . . .. pretending that the aetiology of common diseases like CHD, cancer, diabetes and psychiatric disorders are caused by the independent actions of multiple agents is deterring progress [80].

He went on to call for:

"research that reflects the reality of the problem" and notes that "a reductionist approach that has no interest in complexity discourages imaginative solutions . . . we need an academic environment that puts greater value on how the parts are put together".

Such a systems approach to multiple and cumulative stressors seems to be largely absent from much research and risk assessment of EMF. Recent progress in dealing with cumulative stressors in the chemical field may be of use to EMF scientists [81].

12. Towards transparency in evaluating "weight of evidence"

Since 1965 overall evaluations of scientific evidence for policy making on health hazards has often, implicitly or explicitly, been based on the nine, "Bradford Hill Criteria", which Bradford Hill actually called "features" of evidence [51]. These were produced in response to the smoking and health controversy of the 1960s.

One of the apparently more robust of the nine "criteria", consistency of research results, which is a much discussed issue in the current EMF debate, may not be so robust in the context of multi-causality, complexity and gene/host variability.

Prof. Needleman, who provided the first of what could be called the second generation of early warnings on lead in petrol in 1979, has subsequently observed that:

Consistency in nature does not require that all or even a majority of studies find the same effect. If all studies of lead showed the same relationship between variables, one would be startled, perhaps justifiably suspicious [82].

It follows that the *presence* of consistency of results between studies on the same hazard can provide some of the robust evidence needed to establish a causal link, but the *absence* of such consistency may not provide very robust evidence for the absence of a real association. In other words, the "criterion" of consistency is asymmetrical, like most of the other Bradford Hill "criteria".

This is relevant to the current position with EMF where consistent research results are not generally available. Such inconsistency is to be expected, particularly at this relatively early stage in the complex biological and physical story of EMF.

There is great scope for legitimate differences of view about this and other implications of the complexity, uncertainty and ignorance that characterise the EMF debate. Judgements need to be made, for example, about the weights to be placed on the presence or absence of features of the evidence, such as consistent research results, mechanisms of action, and animal evidence. There is therefore likely to be wide divergences of scientific opinions between different groups of scientists who evaluate the same stock of scientific knowledge during their risk assessments.

For example, in 2000, the UK National Radiological Protection Board set up the Stewart Committee to evaluate the evidence on mobile phones. It concluded that the evidence for safety was not great; that the evidence for harm was weak, but that this was to be expected at this early stage in the history of mobile phones; that the numbers of people, especially young people, exposed was widespread and rising; and that the precautionary principle was relevant, and justified the recommendation that mobiles phones ought not be used by children under 16, except in emergencies [5].

During the same year, a radiation advisory Committee under the Dutch Health Council, comprising similarly qualified scientists, evaluated the same stock of knowledge and concluded that the evidence for safety was robust; that the evidence for harm to RF exposure was largely absent; that children were not more sensitive to RF exposures from mobile phones than adults; and that the precautionary principle was not relevant: no action on exposure reduction was therefore justified [83].

In order to tease out the different and largely hidden assumptions and inferential rules adopted by the two committees, the EEA organised a workshop in May 2008 at which representatives of the two committees explained how they came to such divergent opinions. They were joined by scientists who had produced different evaluations of essentially the same knowledge in three other case studies: ELF from power lines; the plastics chemical, bisphenyl A; and pesticides spray drift.

A brief report summarising the EEA workshop, and containing an eighteen-point checklist that identifies the main reasons for such divergences of view is now available [84].

There appears to be very few risk assessments of EMF that are transparent about how their largely implicit assumptions, judgements and rules of inference affected their conclusions.

An exception is the Californian Department of Health Services evaluation of the possible risks from ELF power line exposures [60]. This report was transparent about its graduated approach to strengths of evidence, about the weights that the individual scientist involved in the assessment placed on different types of evidence, and their types of argumentation and their rules of inference. The assessment was longer and more resource consuming than other EMF risk assessments but its transparency, and stakeholder involvement in agreeing the approach to evaluating the evidence, seems to have produced a more socially and scientifically robust assessment. The recent report from the US National Academy of Sciences on Risk Assessment strongly recommends such transparency and stakeholder involvement, especially at the crucial problem framing stage [65].

13. Conclusion

The successful application of available scientific knowledge and of the precautionary principle to public policy-making on health and environment involves several issues that have been identified in, or have arisen from, debates over some late lessons from early warnings that the EEA has identified. Such issues include the contingent nature of knowledge; approaches to uncertainty, ignorance and "surprises"; appropriate strengths of evidence for policy actions; the biases in the environmental health sciences; public participation in risk analysis and in choices over innovation pathways; and the need for more realism and transparency in the evaluation of evidence about complex ecological and biological realities.

These issues are particularly relevant to the potential hazards that are now emerging from, inter alia, nanotechnology, where scientific ignorance predominates [85]; from the non-ionising radiations arising from the use of mobile phones and power lines; and from endocrine disrupting substances. Such issues require new approaches that, inter alia, involve elements of what has been called post normal science [86].

The capacity of "homo sapiens" (who should perhaps be called, with less hubris, "homo stupidus" as few, if any other species, consciously destroy their habitats) to foresee and forestall disasters, appears to be limited, as the EEA reports on late lessons illustrate.

Societies could, however, with more humility in the face of uncertainty and ignorance, heed the late lessons and, aided by a wider, yet wise application of the precautionary principle, anticipate and minimise hazards. In so doing they would stimulate more participatory risk analysis and governance; the use of more realistic and transparent systems science; and the development of more socially robust and technologically diverse technological and social innovations.

Three main scenarios seem to face us with EMF, particularly with the RF from mobile phones. The first is similar to the case studies in the EEA reports on late lessons, where much avoidable harm was not prevented. The second is where precautionary actions to reduce EMF exposures avert much potential harm, whilst stimulating more sustainable innovation in the production and use of mobile phone technologies and energy systems. And the third is where such precautionary actions to reduce exposures are taken but they turn out to have been unnecessary, if reasonable, given the state of knowledge today. The choice is ours: to act or not to act, as Shakespeare might have said.

Disclaimer

The views expressed are those of the author and do not represent the views of the EEA or its Management Board. The author has no competing financial interest in the matters dealt with.

References

[1] EEA, "Late Lessons from Early Warnings: The Precautionary Principle 1896–2000", European Environment Agency, Copenhagen, Denmark, 2001.

[2] I. Aslaksen, B. Natvig, I. Nordal, Environmental risk and the precautionary principle: "Late Lessons from Early Warnings applied to Genetically Modified Plants", J. Risk Res. 9 (3) (2006) 205–224.

[3] T. Traavik, L. Li Ching, Biosafety First: Holistic approaches to Risk and Uncertainty in Genetic Engineering and Genetically Modified Organisms", Tapir Academic Press, 2007.

[4] Royal Society, Nanoscience and Nanotechnologies: Opportunities and Uncertainties, London, 2003. Available from: <http://www.nanotec.org.uk/finalReport.htm>.

[5] T. Stewart, Report on mobile phones and health, IEGMP, NRPB, 2000. Available from: <http://www.iegmp.org.uk/report/text.htm>.

[6] Mobile Phones and Health, IEGMP Reports, NRPB, 2004. Available from: <http://www.hpa.org.uk/radiation/publications/documents_of_nrpb/pdfs/doc_15_5.pdf>.

[7] WHO, Global Assessment of the State-of-the-Science of Endocrine Disruptors, World Health Organization, Geneva, 2002. Available from: <http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/print.html>.

[8] D. Gee, Late lessons from early warnings: towards realism and precaution with endocrine disrupting substances, Environ. Health Perspect. 114 (Suppl. 1) (2006) 152–160.

[9] Chemtrust, Effects of Pollutants on the Reproductive Health of Male Vertebrate Wildlife Males Under Threat, Gwynne Lyons, London, 2008.

[10] J. Graham, Europe's Precautionary Principles: promise and pitfalls, J. Risk Res. 5 (4) (2002) 375.

[11] M. Planck, Scientific Autobiography and Other Papers, Philosophical Library, New York, 1949.

[12] IPPC, Uncertainty Guidance note for authors of the Fourth Assessment report, Geneva, 2005.

[13] ICNIRP, Guidelines for limiting exposures to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz), Health Physics, 74, Nos. 494–522, 1998; p. 496.

[14] J. Swanson, L. Kheifets, Biophysical mechanisms: a component in the weight of evidence for health effects of power frequency electric and magnetic fields, Rad. Res. 165 (2006) 470–478.

[15] WHO, Environmental Health Criteria 238, Extremely Low Frequency Fields, 2007.

[16] W.R. Adey, Potential therapeutic applications of non-thermal electromagnetic fields: ensemble organisation of cells in tissue as a factor in

biological field sensing, in: P.J. Rosch, M.S. Markov (Eds.), Bioelectromagnetic Medicine, 2004.
[17] C. Brauner, Electrosmog—a Phantom Risk, Swiss Re.
[18] D.L. Henshaw, M.J. O'Carroll, Response to Draft Opinion of SCENIHR on EMF, University of Bristol, 2006.
[19] M. Blank, Health Risk of Electromagnetic Fields: Research on the Stress Response, in Bioinitiative Report: a Rationale for a Biologically based Public Exposure Standard for Electromagnetic Fields (ELF and RF), 2007. Available from: <http://www.bioinitiative.org>.
[20] T. Kuhn, The Structure of Scientific Revolutions, University of Chicago Press, 1962.
[21] O'Carroll, Application of the Precautionary Principle to EMF, Paper to the WHO/EC/NIEHS Workshop, Luxembourg, 24–26th February 2003.
[22] D.L. Henshaw, R.J. Reiter, Do magnetic fields cause increased risk of childhood leukemia via melatonin disruption? A review, Bioelectromagn. Suppl. 7 (2005) S86–S97.
[23] Swedish Environmental Protection Agency, 4th Report on EMF, 2007, p. iii.
[24] IARC, Non-ionising radiation. Part 1. Static and extremely low frequency (ELF) electric and magnetic fields, in: International Agency for Research on Cancer Monographs on the evaluation of carcinogenic risks to humans, Lyon, vol. 80, 2002.
[25] X. Shen, Y. Yang, X. Jin, C. Yan, Y. Tian, J. Tang, Case-only study of interactions between DNA repair genes (*hMLH1, APEX1, MGMT, XRCC1* and *XPD*) and low-frequency electromagnetic fields in childhood acute leukaemia, Leukemia Lyphoma (December) (2008).
[26] B. Lambert, Radiation: Early Warnings, Late Effects, Chapter 3 in Late Lessons from Early Warnings, EEA, 2001.
[27] SCENIHR, Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Opinion on Possible Effects of Electromagnetic Fields (EMF) on Human Health, March 2007. Available from: <http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_007.pdf>.
[28] The Controversy about a Possible relationship between Mobile Phone Use and Cancer, Environmental Health Perspectives online, 26 September 2008.
[29] L. Hardell, M. Carlberg, F. Sodrqvist, K.H. Mild, Meta-analysis of long term mobile phone use and the association with brain tumours, Int. J. Oncol. 32 (2008) 1097–1103.
[30] L.E. Edqvist, K.B. Pedersen, Antimicrodials as Growth Promotors: resistance to Common Sense, Chapter 9 in Late Lessons from Early Warnings, EEA, 2001.
[31] M.M. Swann Report, Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine, HMSO, London, 1969.
[32] Case T-13/99 Pfizer ECR II-3305 and in Case T-70/99, Alpharma, ECR, II-3495, September 11, 2002.
[33] Bioinitiative Report: a Rationale for a Biologically based Public Exposure Standard for Electromagnetic Fields (ELF and RF), 2007. Available from: <http://www.bioinitiative.org>.
[34] EEA, Radiofrequency Electromagnetic Fields: EEA Commentary on the Evaluation of the Evidence, March; EEA web item September 17th 2007, Radiation Risk from everyday devices assessed, Copenhagen (2007/08).
[35] N. Stern, The Economics of Climate Change, UK Treasury, 2006.
[36] BCPT, Special Issue on Prenatal Programming and Toxicity, in: P. Grandjean (Guest Ed.), Basic and Clinical Pharmacology & Toxicology, 102, No. 2, February 2008.
[37] Grandjean et al., The Faroes Statement: Human Health Effects of Developmental Exposure to Chemicals in Our Environment, in BCPT, 2008.
[38] D. Gee, Establishing Evidence for Early Action: the prevention of reproductive and developmental harm, Basic Clin. Pharmacol. Toxicol. 102 (2) (2008) 257–266.
[39] Plato's Apology 1.21, "So I am likely to be wiser than he to this small extent, that I do not think I know what I do not know.".
[40] D. Brewster (Ed.), Memoirs of Newton, vol. 2, 1855 (Chapter 27).
[41] A. Stirling, On science and precaution in the management of technological risk. Final summary report Technological Risk and Uncertainty project, European Scientific Technology Observatory, EC Forward Studies Unit, Brussels, 1999.
[42] D. Orrell, The Future of Everything; the Science of Prediction, Thunder's Mouth Press, New York, 2007.
[43] N.N. Taleb, The Black Swan: the Impact of the Highly Improbable, Penguin, 2008.
[44] D. Santillo, P. Johnston, W.J. Langston, Tributyltin (TBT) antifoulants: a tale of ships, snails and imposex, chapter 13 in Late Lessons from Early warnings, EEA, 2001.
[45] M. Stroebe, M. Scheringer, K. Hungerbuhler, Measures of overall persistence and the temporal remote state, Environ. Sci. Technol. 38 (2004) 5665–5673.
[46] UN, World Charter for Nature, UN General Assembly 37th Session (UN/GA/RES/37/7), New York, 1982.
[47] Marine Pollution Bulletin 34 (9) (1997) 680–681.
[48] A. Trouwborst, The Precautionary Principle in General International Law: Combating the Babylonian Confusion, RECEIL 16 (2) (2007) 185–195.
[49] Treaty establishing the European Community (consolidated text), Official Journal C 325 of 24 December 2002. Available from: <http://europa.eu.int/eur-lex/lex/en/treaties/dat/12002E/pdf/12002E_EN.pdf>.
[50] N. De Sadeleer, Implementing the Precautionary Principle: Approaches from the Nordic Countries, EU, and USA, Earthscan.
[51] A. Bradford Hill, The environment and disease: association or causation? in: Proceedings of the Royal Society of Medicine, vol. 58, 1965, pp. 295–300.
[52] General Food Law regulation, EC No. 178/2002, Official Journal of the EU, L31, 0.02.2002, Luxembourg.
[53] T. Christoforou, Science, law and precaution in dispute resolution on health and environmental protection: what role for scientific experts?, in: Le commerce international des organisms genetiquement modifies, Centre d'Etudes et de Recherches Internationales et Communautaires, Universite d'Aix-Marseille, 2002, p. 111.
[54] WHO, Declaration of Fourth Ministerial Conference on Environment and Health, Budapest, Hungary, 23–25 June 2004. Available from: <http://www.euro.who.int/document/e83335.pdf>.
[55] WHO, Dealing with uncertainty – how can the precautionary principle help protect the future of our children? Working paper for the Fourth Ministerial Conference on Environment and Health, Budapest, Hungary, 23–25 June 2004. Available from: <http://www.euro.who.int/document/hms/edoc11.pdf>.
[56] Health Council of the Netherlands, Prudent Precaution, 2008.
[57] European Commission, Communication from the Commission on the Precautionary Principle, COM, Brussels, 2000, p. 1.
[58] V.J. Cogliano, The IARC Monographs: a resource for Precaution and Prevention, a Commentary on the Editorial by Martuzzi on "The Precautionary Principle: in Action for Public Health", 2007, pp. 569–574. Available from: <http://oem.bmj.com>.
[59] IPCC, Intergovernmental Panel on Climate Change, Second, Third, and Fourth Assessment Reports, 1995, 2001, 2007. Available from: <http://www.ipcc.ch/pub/reports.htm>.
[60] R. Neutra, in: P.M. Wiedemann, H. Schutz (Eds.), Lessons from the California Electromagnetic Field Risk Assessment of 2002, The role of evidence in Risk Characterisation: Making sense of Conflicting Data, Wiley–VCH, Germany, 2007.
[61] US Presidential/Congressional Commission on Risk Assessment & Risk Management, Framework for Environmental Health Risk Assessment, Final Report, vol. 1, 1997. Available from: <http://www.riskworld.com/Nreports/1997/risk-rpt/pdf/EPAJAN.PDF>, Management.
[62] Royal Commission on Environmental Pollution Environmental Standards, The Stationary Office, London, 1998.
[63] German Advisory Council on Global Change, Strategies for Managing Global Environmental Risks, 2001.

[64] Codex Alimentarius Commission, Principles for Risk Analysis for Food Safety, FAO/WHO, Rome, 2007.
[65] NAS, Science and Decisions: Advancing Risk Assessment, Washington, 2008. Available from: <http://www.nap.edu/catalog/12209.html>.
[66] E. Millstone et al., JRC/IPTS, Risk Assessment Policies: Differences across jurisdictions, EU Joint Research Centre, Institute for Prospective Technological Studies, Seville, 2007.
[67] T. Christoforou, The precautionary principle and democratising expertise: a European legal perspective, Science and Public Policy 30, Surrey, England, No. 3, June 2003, pp. 205–221.
[68] J. Wilsdon, R. Willis, See-through Science – Why Public Engagement needs to Move Upstream, Demos, London, 2004.
[69] Wynne et al., Taking European Knowledge Society Seriously, Report of the Expert Group on Science and Governance to the Science, Economy and Society Directorate, Directorate-General for Research, European Commission, 2007.
[70] A. Pereira, S.G. Vaz, S. Tognetti (Eds.), Interfaces between Science and Society, Greenleaf Publishing.
[71] Netherlands Environmental Assessment Agency, Stakeholder Participation Guidance, Main Document, 2008.
[72] M. O'Carroll, Precautionary approaches and the SAGE experience, paper to Radiation Research Trust Conference: EMF and Health – A Global Issue, London, 8–9 September 2008.
[73] A. Mazur, True Warnings and False Alarms. Evaluating Fears about the Health Risks of Technology, 1948–1971, Resources for the Future, Washington, 2004.
[74] S.F. Hansen, M.P. Krayer von Krauss, J. Tickner, Categorising Mistaken False Positives in Regulation of Human and Environmental Health Risk Anal. 27 (1) (2007).
[75] P. Grandjean, M. Soffriti, F. Minardi, J. Brazier, The Precautionary Principle: Implications for Research and Prevention in Environmental and Occupational Health European Journal of Oncology Library, vol. 2, European Ramazzini Foundation, Bologna, Italy, 2003.
[76] Max Planck, Lecture on the Law of Causality and Free Will, February 1927.
[77] WHO, Extremely Low Frequency Fields, Environmental Health Criteria, No. 238, WHO, 2007, p. 274.
[78] D. Ibarreta, S.H. Swann, The DES story: long term consequences of prenatal exposure, Chapter 8 in Late Lessons from Early Warnings, EEA, 2001.
[79] P. Vineis, A self-fulfilling prophecy: are we underestimating the role of the environment in geno–environment interaction research? Int. J. Epidemiol. 33 (2004) 945–946.
[80] C.F. Sing, J.H. Stengard, S.L.R. Kardia, Dynamic relationships between the Genome and Exposures to environments as causes of common human diseases, Chapter in Nutrigenetics and Nutrigenomics World Review of Nutrition and Diet, Basel, Karger, vol. 93, 2004, pp. 77–91.
[81] US National Academy of Sciences, Cumulative Risk Assessment of Phthalates and Related Chemicals, National Academy of Sciences, 87 US Surgeon General (1964), Smoking and Health, Department of Health and Human Sciences, Washington, 2008.
[82] H.L. Needleman, Making Models of Real World events: the use and abuse of inference, Neurotoxicol. Teratol. 17 (3) (1995).
[83] Health Council of the Netherlands, Mobile Phones: an Evaluation of Health Effects, 2002.
[84] EEA, paper summarizing the workshop on Evaluating Evidence, Copenhagen, May 2008.
[85] Royal Commission on Environmental Pollution, Novel Materials in the Environment: the Case of Nanotechnology, The Stationary Office, London, 2008.
[86] S. Funtowicz, J. Ravetz, in: S. Krimsky, D. Golding (Eds.), Three Types of Risk Assessment and the Emergence of Post-Normal Science: In Social Theories of Risk, Praeger, Westport, 1992, pp. 251–273.

ELSEVIER

Pathophysiology 16 (2009) 71–78

PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Electromagnetic fields stress living cells

Martin Blank [a,*], Reba Goodman [b]

[a] Department of Physiology, Columbia University, New York, NY, USA
[b] Department of Pathology, Columbia University, New York, NY, USA

Received 30 January 2009; accepted 30 January 2009

Abstract

Electromagnetic fields (EMF), in both ELF (extremely low frequency) and radio frequency (RF) ranges, activate the cellular stress response, a protective mechanism that induces the expression of stress response genes, e.g., HSP70, and increased levels of stress proteins, e.g., hsp70. The 20 different stress protein families are evolutionarily conserved and act as 'chaperones' in the cell when they 'help' repair and refold damaged proteins and transport them across cell membranes. Induction of the stress response involves activation of DNA, and despite the large difference in energy between ELF and RF, the same cellular pathways respond in both frequency ranges. Specific DNA sequences on the promoter of the HSP70 stress gene are responsive to EMF, and studies with model biochemical systems suggest that EMF could interact directly with electrons in DNA. While low energy EMF interacts with DNA to induce the stress response, increasing EMF energy in the RF range can lead to breaks in DNA strands. It is clear that in order to protect living cells, EMF safety limits must be changed from the current thermal standard, based on energy, to one based on biological responses that occur long before the threshold for thermal changes.

Keywords: DNA; Biosynthesis; Electromagnetic fields; ELF; RF

1. Electromagnetic fields (EMF) alter protein synthesis

Until recently, genetic information stored in DNA was considered essentially invulnerable to change as it was passed on from parent to progeny. Mutations, such as those caused by cosmic radiation at the most energetic end of the EM spectrum, were thought to be relatively infrequent. The model of gene regulation was believed to be that the negatively charged DNA was tightly wrapped up in the nucleus with positively charged histones, and that most genes were 'turned off' most of the time. Of course, different regions of the DNA code are being read more or less all the time to replenish essential proteins that have broken down and those needed during cell division.

New insights into the structure and function of DNA have resulted from numerous, well-done laboratory studies. The demonstration that EMF induces gene expression and the synthesis of specific proteins [1,2] generated considerable controversy from power companies, government agencies, physicists, and most recently, cell phone companies. Physicists have insisted that the reported results were not possible because there was not enough energy in the power frequency range (ELF) to activate DNA. They were thinking solely of mechanical interaction with a large molecule and not of the large hydration energy tied up in protein and DNA structures that could be released by small changes in charge [3]. Of the biologists who accepted such results [4], most thought that the EMF interaction originated at, and was amplified by, the cell membrane and not with DNA.

It is now generally accepted that weak EMF in the power frequency range can activate DNA to synthesize proteins. An EMF reactive sequence in the DNA has been identified [5] and shown to be transferable to other gene promoters [6]. This DNA sequence acts as an EMF sensitive antenna

Abbreviations: EMF, electromagnetic fields; Hz, hertz; ELF, extremely low frequency; RF, radio frequency; MAPK, mitogen activated protein kinase; ERK1\2, extracellular signal regulated kinase; JNK, c-Jun-terminal kinase p38MAPK; SAPK, stress activated protein kinase; NADH, nicotinamide adenine dinucleotide dehydrogenase; ROS, reactive oxygen species.

* Corresponding author at: Department of Physiology, Columbia University, 630 West 168 Street, New York, NY 10032, USA. Tel.: +1 212 305 3644; fax: +1 212 305 5775.
E-mail address: mb32@columbia.edu (M. Blank).

0928-4680/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2009.01.006

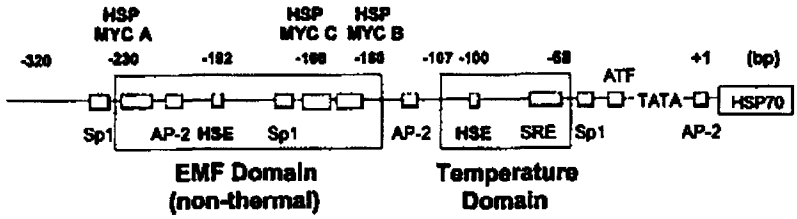

Fig. 1. Diagram of the HSP70 promoter showing the two different DNA sequences that have been identified as activated by EMF (non-thermal) and by thermal stimuli, respectively. The EMF domain contains three nCTCTn consensus sequences (electromagnetic response elements; EMRE), and differs from the consensus sequence (nGAAn) in the temperature or thermal domain.

that responds to EMF when transfected into reporter genes. Research at the more energetic levels of power frequency [7] and in the RF [8] ranges has shown that exposure to EMF can lead to breaks in the DNA strands. Therefore, DNA can no longer be considered unaffected by environmental EMF levels. It can be activated and damaged by EMF at levels that are considered safe [9]. The vulnerability of DNA to environmental influences and the possible dangers associated with EMF, had been underscored by discovery of EMF activation of the cellular stress response in the ELF range [10,11]. The cellular stress response is an unambiguous signal by the cell that EMF is potentially harmful.

2. Physiological stress and cellular stress

Discussions of physiological stress mechanisms usually describe responses of the body to pain, fear, 'oxygen debt' from muscle overexertion. These responses are mediated by organ systems. For example, the nervous system transmits action potentials along a network of nerves to cells, such as adrenal glands, that release rapidly acting agents such as epinephrine and norepinephrine and slower acting mineralocorticoids. These hormones are transported throughout the body by the circulatory system. They mobilize the defenses to cope with the adverse conditions and enable the body to 'fight or flee' from the noxious stimuli. The defensive actions include changes in heart rate, breathing rate, muscle activity, etc.

In addition to the responses of organ systems, there are protective mechanisms at the cellular level known as the cellular stress response. These mechanisms are activated by damage to cellular components such as DNA and protein [12], and the responses are characterized by increased levels of stress proteins [13] indicating that stress response genes have been upregulated in response to the stress.

The first stress response mechanism identified was the cellular reaction to sharp increases in temperature [14] and was referred to as 'heat shock', a term that is still retained in the nomenclature of the protective proteins, the hsps, heat shock proteins. Stress proteins are designated by the prefix 'hsp' followed by a number that gives the molecular weight in kilodaltons. There are about 20 different protein families ranging in molecular weight from a few kilodaltons to over 100 kD, with major groups of proteins around 30 kD, 70 kD and 90 kD.

Research on the 'heat shock' response has shown that hsp synthesis is activated by a variety of stresses that are potentially harmful to cells, including physical stimuli like pH and osmotic pressure changes, as well as chemicals such as alcohol and toxic metal ions like Cd^{2+}. EMF is a recent addition to the list of physical stimuli. It was initially shown in the power frequency (extremely low frequency, ELF) range [13], but shortly afterwards, radio frequency (RF) fields [15] and amplitude modulated RF fields [16] were shown to activate the same stress response.

Studies of stress protein stimulation by low frequency EMF have focused on a specific DNA sequence in the gene promoter that codes for hsp70, a major stress protein. Synthesis of this stress protein is initiated in a region of the promoter (see Fig. 1) where a transcription factor known as heat shock factor 1 (HSF-1) binds to a heat shock element (HSE). This EMF sensitive region on the HSP70 promoter is upstream from the thermal domain of the promoter and is not sensitive to increased temperature. The binding of HSF-1 to HSE occurs at −192 in the HSP70 promoter relative to the transcription initiation site. The EMF domain contains three nCTCTn myc-binding sites −230, −166 and −160 relative to the transcription initiation site and upstream of the binding sites for the heat shock (nGAAn) and serum responsive elements [5,6,17,18]. The electromagnetic response elements (EMREs) have also been identified on the c-*myc* promoter and are also responsive to EMF. The sensitivity of the DNA sequences, nCTCTn, to EMF exposures has been demonstrated by transfecting these sequences into CAT and Luciferase reporter genes [6]. Thus, the HSP70 promoter contains different DNA regions that are specifically sensitive to different stressors, thermal and non-thermal.

Induction of increased levels of the major stress protein, hsp70, by EMF is rapid, within 5 min. Also it occurs at extremely low levels of energy input, 14 orders of magnitude lower than with a thermal stimulus [10]. The far greater sensitivity to EMF than to temperature change in elevating the protective protein, hsp70, has been demonstrated to have potential clinical application, preventing injury from ischemia reperfusion [19–21]. George et al. [22] have shown the non-invasive use of EMF-induced stress proteins improved hemodynamic parameters during reperfusion

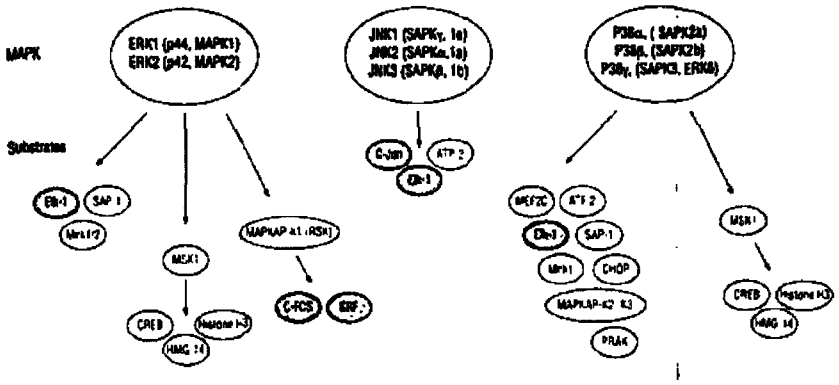

Fig. 2. The four mitogen activated protein kinase (MAPK) signaling cascades identified to date are: extracellular signal regulated kinase 1/2 (ERK), c-Jun-terminal kinase (JNK), p38MAPK and stress activated protein kinase (SAPK). Elements of the three MAPkinase pathways that have been identified as activated by EMF are shown as the shaded circles.

following ischemia. This effect occurred in the absence of measurable increased temperature.

3. EMF interaction with signaling pathways

EMF penetrate cells unattenuated and so can interact directly with the DNA in the cell nucleus, as well as other cell constituents. However, biological agents are impeded by membranes and require special mechanisms to gain access to the cell interior. Friedman et al. [23] have demonstrated that the initial step in transmitting extracellular information from the plasma membrane to the nucleus of the cell occurs when NADH oxidase rapidly generates reactive oxygen species (ROS). These ROS stimulate matrix metalloproteinases that allow them to cleave and release heparin binding epidermal growth factor. This secreted factor activates the epidermal growth receptor, which in turn activates the extracellular signal regulated kinase 1\2 (ERK) cascade. The ERK cascade is one of the four mitogen-activated protein kinase (MAPK) signaling cascades that regulate transcriptional activity in response to extracellular stimuli. The elements of the three MAPK signaling cascades implicated in exposures to ELF and RF are highlighted in Fig. 2.

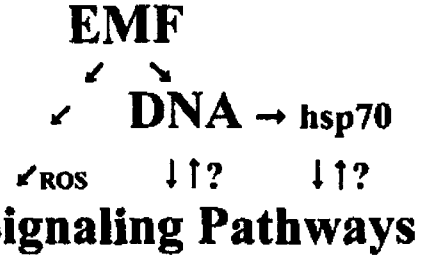

Fig. 3. The signaling pathways and the stress response are activated by EMF. The activation mechanisms discussed in the text are indicated by arrows. In the stress response, DNA activation leads to hsp synthesis and may be due to direct EMF interaction with DNA. The signaling pathways are activated by reactive oxygen species (ROS) that are probably generated by EMF. Possible interactions between the pathways, DNA and hsp are indicated with question marks. In any case, EMF leads to activation of all the processes shown.

The four MAPK cascades are: (1) ERK, (2) c-Jun-terminal kinase (JNK), (3) stress activated protein kinase (SAPK) and (4) p38SAPK. Each of the cascades is composed of three to six tiers of protein kinases, and their signals are transmitted by sequential phosphorylation and activation of the protein kinases in each of the tiers. The result is activation of a large number of regulatory proteins, which include a set of transcription factors, e.g., c-Jun, c-Fos, hsp27 and hsp70. Activation of the stress response is accompanied by activation of specific signal transduction cascades involved in regulating cell proliferation, differentiation and metabolism [24–26]. The MAPK pathways have been characterized in several cell types [24,27–30]. Exposure to non-thermal ELF as well as thermal RF affects the expression of many cellular proteins [23–25] (Fig. 3).

The elevated expression of these protein transcription factors participate in the induction of various cellular processes, including several that are affected by cell phones, e.g., replication and cell-cycle progression [25,31] and apoptosis [32]. RF fields have been shown to activate specific transcription factor binding that stimulate cell proliferation and induce stress proteins [25,33]. It has been reported [31] that within 10 min of cell phone exposures, two MAPKinase cascades, p38 and ERK1\2, are activated. Both ELF and RF activate the upregulation of the HSP70 gene and induction of elevated levels of the hsp70 protein. This effect on RNA transcription and protein stability is controlled by specific protein transcription factors that are elements of the mitogen MAPK cascade.

EMF also stimulate serum response factor which binds to the serum response element (SRE) through ERK MAPK activation and is associated with injury and repair *in vivo* and *in vitro*. The SRE site is on the promoter of an early response gene, c-fos, which under specific cellular circumstances has oncogenic properties. The c-fos promoter is EMF-sensitive; a 20 min exposure to 60 Hz 80mG fields significantly increases c-*fos* gene expression [34]. The SRE accessory protein,

Elk-1, contains a growth-regulated transcriptional activation domain. ERK phosphorylation potentiates Elk-1 and transactivation at the c-*fos* SRE [29].

During the past twenty years, the growing use of cellular phones has aroused great concern regarding the health effects of exposure of the brain to 900 MHz RF waves. Despite claims that the energy level is too low to induce changes in DNA and that the devices are safe, the non-thermal effects that have been demonstrated at both ELF and RF exposure levels can cause physiological changes in cells and tissues even at the level of DNA. Finally, it should be mentioned that some of the pathways described in this section also have roles in protein synthesis via RNA polymerase III, an enzyme in oncogenic pathways [35] and could, therefore, provide a mechanistic link between cancer and EMF exposure.

4. Cells affected by the stress response

Reviews on EMF and the stress response have appeared for the ELF range [13] and for the RF range [36]. The most recent review was published online in section 7 of the Bioinitiative Report [9], and it summarized both ELF and RF studies, mainly at frequencies 50 Hz, 60 Hz, 900 MHz and 1.8 GHz. The citations in that review were not exhaustive, but the different frequencies and biological systems represent the diversity of results on stimulation of DNA and stress protein synthesis in many different cells. It is clear that the stress response does not occur in reaction to EMF in all types of cells, and sometimes because of the use of tissue cultured cell lines, even the same cell line can give opposite results in the same laboratory [37].

Many different types of cells have been shown to respond to EMF, both *in vivo* and *in vitro*, including epithelial, endothelial and epidermal cells, cardiac muscle cells, fibroblasts, yeast, *E. coli*, developing chick eggs, and dipteran cells (see Bioinitiative Report [9], section 7). Tissue cultured cells are less likely to show an effect of EMF, probably because immortalized cells have been changed significantly to enable them to live indefinitely in unnatural laboratory conditions. This may also be true of cancer cells, although some (e.g., MCF7 breast cancer cells) have responded to EMF [38,39], and in HL60 cells, one cell line responds to EMF while another does not [24]. Czyz et al. [16] found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not.

A broad study of genotoxic effects (i.e., DNA damage) in different kinds of cells [40] found no effects with lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Other studies [41,42] have also found that the blood elements, such as lymphocytes and monocytes are natural cells that have not responded. Since mobile cells can easily move away from a stress, there would be little selective advantage and evolutionary pressure for developing the stress response. The lack of response by skeletal muscle cells is related to the need to desensitize the cells to excessive heating during activity. Unlike slow muscle fibers that do synthesize hsp70, cells containing fast muscle fibers do not synthesize hsp70 to protect them from over-reacting to the high temperatures reached a during activity.

Table 1
Biological thresholds in the ELF range.

Biological system	Threshold (μT)[a]	Reference
Acceleration of reaction rates		
Na,K-ATPase	0.2–0.3	Blank and Soo [49]
cytochrome oxidase	0.5–0.6	Blank and Soo [43]
ornithine decarboxylase	~2	Mullins et al. [58]
malonic acid oxidation	<0.5	Blank and Soo [59]
Biosynthesis of stress proteins		
HL60, Sciara, yeast,	<0.8	Goodman et al. [11]
breast (HTB124, MCF7)	<0.8	Lin et al. [39]
chick embryo (anoxia)	~2	*DiCarlo et al. [60]*
Breast cancer (MCF7) cell growth		
block melatonin inhibition	0.2 < 1.2	Liburdy et al. [38]
Leukemia epidemiology	0.3–4	Ahlbom et al. [61] Greenland et al. [62]

[a] The estimated values are for departures from the baseline, although Mullins et al. (1999) and DiCarlo et al. (2000) generally give inflection points in the dose–response curves. The leukemia epidemiology values are not experimental and are listed for comparison.

5. EMF–DNA interaction mechanisms: electron transfer

The biochemical compounds in living cells are composed of charges and dipoles that can interact with electric and magnetic fields by various mechanisms. An example discussed earlier is the generation of reactive oxygen species (ROS) in activation of the ERK signaling cascade. The cellular stress response leading to the synthesis of stress proteins is also activated by EMF. However, the specific reaction is not known, except that it is stimulated by very weak EMF. For this reason, our focus has been on molecular processes that are most sensitive to EMF and that could cause the DNA to come apart to initiate biosynthesis. We have suggested that direct EMF interaction with electrons in DNA is likely for the following reasons:

- The largest effects of EMF would be expected on electrons because of their high charge to mass ratio. At the sub-atomic level, one assumes that electrons respond instantaneously compared to protons and heavier atomic nuclei, as in the Born-Oppenheimer Approximation. The very low field strengths and durations that activate the stress response and other reactions (Table 1) suggest interaction with electrons, and make ion-based mechanisms unlikely.
- Weak ELF fields have been shown to affect the rates of electron transfer reactions [43,44]. A 10 μT magnetic field exerts a very small force of only $\sim 10^{-20}$ N on a unit charge,

but this force can move an isolated electron more than a bond length, ~1 nm, in ~1 nanosecond.
- There is a specific EMF responsive DNA sequence that is associated with the response to EMF (Fig. 1), and that retains this property when transfected
- Displacement of electrons in DNA would cause local charging that has been shown to lead to disaggregation of biopolymers [45].
- As the energy in an EMF stimulus increases, there is an increase in single strand breaks, followed by double strand breaks, suggesting an interaction with EMF at all energy levels [46].

Effects of EMF on electrons in chemical reactions were detected indirectly in studies on the Na,K-ATPase [47], a ubiquitous enzyme that establishes the normal Na and K ion gradients across cell membranes. Electric and magnetic fields, each accelerated the reaction only when the enzyme was relatively inactive. It is reasonable to assume that the threshold response occurs when the same charge is affected by the two fields, so the velocity (v) of the charge (q) could be calculated from these measurements and its nature determined. Assuming both fields exert the same force at the threshold, the electric (E) and the magnetic (B) forces should be equal.

$$F = qE = qvB. \quad (1)$$

From this $v = E/B$, the ratio of the threshold fields, and by substituting the measured thresholds [48,49], $E = 5 \times 10^{-4}$ V/m and $B = 5 \times 10^{-7}$T (0.5 μT), we obtain $v = 10^3 m/s$. This very rapid velocity, similar to that of electrons in DNA [50], indicated that electrons were probably involved in the ion transport mechanism of the Na,K-ATPase [47]. An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the ELF field has had a chance to change. This means that a low frequency sine wave signal is effectively a repeated DC pulse. This is true of all low frequency effects on fast moving electrons.

Studies of effects of EMF on electron transfer in cytochrome oxidase, ATP hydrolysis by the Na,K-ATPase, and the Belousov–Zhabotinski (BZ) redox reaction, have led to certain generalizations:

- EMF can accelerate reaction rates, including electron transfer rates
- EMF acts as a force that competes with the chemical forces in a reaction. The effect of EMF varies inversely with the intrinsic reaction rate, so EMF effects are only seen when intrinsic rates are low. (This is in keeping with the therapeutic efficacy of EMF on injured tissue, while there is usually little or no effect on normal tissue.)
- Experimentally determined thresholds are low (~0.5 μT) and comparable to levels found by epidemiology. See Table 1.
- Effects vary with frequency, with different optima for the reactions studied: The two enzymes showed broad frequency optima close to the reaction turnover numbers for Na,K-ATPase (60 Hz) and cytochrome oxidase (800 Hz), suggesting that EMF interacted optimally when in synchrony with the molecular kinetics. This is not true for EMF interactions with DNA, which are stimulated in both ELF and RF ranges and do not appear to involve electron transfer reactions with well-defined kinetics.

Probably the most convincing evidence for a frequency sensitive mechanism that involves stimulation of DNA is activation of protein synthesis in striated muscle. In this natural process, specific muscle proteins are synthesized by varying the rate of the (electrical) action potentials in the attached nerves [51]. The ionic currents of the action potentials that flow along and through the muscle membranes, also pass through the muscle cell nuclei that contain the DNA codes for the muscle proteins. Two frequencies were studied in muscle, high (100 Hz) and low (10 Hz) frequency, corresponding to the frequencies of the fast muscles and slow muscles that have different contraction rates and different muscle proteins. In the experiments, either the fast or slow muscle proteins were synthesized at the high or low frequency stimulation rates corresponding to the frequency of the action potentials. The clear dependence of the protein composition on the frequency of the action potentials indicates a relation between stimulation and activation of DNA in muscle physiology. The process is undoubtedly far more complicated and unlikely to be a simple electron transfer reaction as with cytochrome oxidase. It is more probable that an entire region of DNA coding for a group of related proteins is activated simultaneously.

A mechanism based on electron movement is in keeping with the mV/m electric field and μT magnetic field thresholds that affect the Na,K-ATPase. The very small force on a charge ($\sim 10^{-20}$ N) can affect an electron, but is unlikely to have a direct effect on much more massive ions and molecules, especially if they are hydrated. Ions are affected by the much larger DC electric fields of physiological membrane processes. The low EMF energy can move electrons, cause small changes in charge distribution and release the large hydration energy tied up in protein and DNA structures [3]. Electrons have been shown to move in DNA at great speed [50], and we have suggested that RF and ELF fields initiate the stress response by directly interacting and accelerating electrons moving within DNA [52,53].

A mechanism based on electron movement also provides insight into why the same stress response is stimulated by both ELF and RF even though the energies of the two stimuli differ by orders of magnitude. A typical ELF cycle at 10^2Hz lasts 10^{-2} s and a typical RF cycle at 10^{11} Hz lasts 10^{-11} s. Because the energy is spread over a different number of cycles/second in the two ranges, the energy/cycle is the same in both ELF and RF ranges. Since electron movement occurs much faster than the change of field, both frequencies are seen by rapidly moving electrons as essentially DC pulses. Each cycle contributes to electron movement at both

frequencies, but more rapidly at the higher frequency. The fluctuation of protons between water molecules in solution at a frequency of about 10^{12} Hz [54] gives an indication of the speed of electron movement, and may suggest an upper limit of the frequency in which sine wave EMF act as DC pulses.

6. DNA biology and the EM spectrum

Research on DNA and the stress response has shown that the same biology occurs across divisions of the EM spectrum, and that EMF safety standards based on cellular measures of potential harm should be much stricter. These data also raise questions about the utility of spectrum sub-divisions as the basis for properly assessing biological effects and setting separate safety standards for the different sub-divisions. The frequencies of the EM spectrum form a continuum, and division into frequency bands is only a convenience that makes it easier to assign and regulate different portions of the spectrum for practical uses, such as the different design requirements of devices for EMF generation and measurement. Except for the special case of the visual range, the frequency bands are not based on biology, and the separate bands now appear to be a poor way of dealing with biological responses needed for evaluating safety. The DNA studies indicate the need for an EMF safety standard rooted in biology and a rational basis for assessing health implications.

DNA responses to EMF can be used to create a single scale for evaluation of EMF dose because:

- The same biological responses are stimulated in ELF and RF ranges.
- The intensity of EMF interactions with DNA leads to greater effects on DNA as the energy increases with frequency. In the ELF range, the DNA is only activated to initiate protein synthesis, while single and double strand breaks occur in the more energetic RF and ionizing ranges.

A scale based on DNA biology also makes possible an approach to a quantitative relation between EMF dose and disease. This can be done by utilizing the data banks that have been kept for A-bomb exposure and victims of nuclear accidents, data that link exposure to ionizing radiation and subsequent development of cancer. Utilizing experimental studies of DNA breaks with ionizing radiation, it is possible in principle to relate cancer incidence to EMF exposures. It should be possible to determine single and double strand breaks in a standard preparation of DNA, caused by exposure to EMF for a specified duration, under standard conditions. Although many studies of DNA damage and repair rates under different conditions would be needed, this appears to be a possible experimental approach to assessing the relation between EMF exposure and disease.

7. The stress response and safety standards

Most scientists believe that basic research eventually pays off in practical ways. This has certainly been true of EMF research on the stress response, where EMF stimulated stress proteins have been used to minimize damage to ischemic tissues on reperfusion. However, more importantly, biological effects stimulated by both ELF and RF have shown that the standards used for developing safety guidelines are not protective of cells.

First and foremost, it is important to realize that the stress response occurs in reaction to a potentially harmful environmental influence. The stress response is an unambiguous indication that cells react to EMF as potentially harmful. It is therefore an indication of compromised cell safety, given by the cell, in the language of the cell. The low threshold level of the stress response shows that the current safety standards are much too high to be considered safe.

In general, cellular processes are unusually sensitive to fields in the environment. The biological thresholds in the ELF range (Table 1) are in the range of 0.5–1.0 μT—not very much higher than the ELF backgrounds of ~0.1 μT. The relatively low field strengths that can affect biochemical reactions is a further indication that cells are able to sense potential danger long before there is an increase in temperature.

EMF research has also shown that exposure durations do not have to be prolonged to have an effect. Litovitz et al. [55,56], working with the enzyme ornithine decarboxylase, showed an EMF response when cells were exposed for only 10 s to ELF or ELF modulated 915 MHz, providing that the exposure was continuous. Gaps in the sine wave resulted in a reduced response, and interference with the sine wave in the form of superimposed ELF noise also reduced the response [57]. The interfering effect of noise has been shown in the RF range by Lai and Singh [46], who reported that noise interferes with the ability of an RF signal to cause breaks in DNA strands. The decreased effect when noise is added to a signal is yet another indication that EMF energy is not the critical factor in causing a response. In fact, EMF noise appears to offer a technology for mitigating potentially harmful effects of EMF in the environment.

EMF research has shown that the thermal standard used by agencies to measure safety is at best incomplete, and in reality not protective of potentially harmful non-thermal fields. Non-thermal ELF mechanisms are as effective as thermal RF mechanisms in stimulating the stress response and other protective mechanisms. The current safety standard based on thermal response is fundamentally flawed, and not protective.

Finally, since both ELF and RF activate the same biology, simultaneous exposure to both is probably additive and total EMF exposure is important. Safety standards must consider total EMF exposure and not separate standards for ELF and RF ranges.

[43] M. Blank, L. Soo, Enhancement of cytochrome oxidase activity in 60 Hz magnetic fields, Bioelectrochem Bioenerg 45 (1998) 253–259.
[44] M. Blank, L. Soo, Electromagnetic acceleration of the Belousov-Zhabotinski reaction, Bioelectrochem 61 (2003) 93–97.
[45] M. Blank, L. Soo, Surface free energy as the potential in oligomeric equilibria: prediction of hemoglobin disaggregation constant, Bioelectrochem Bioenerg 17 (1987) 349–360.
[46] H. Lai, N.P. Singh, Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells, Electromagn Biol Med 24 (2005) 23–29.
[47] M. Blank, A proposed explanation for effects of electric and magnetic fields on the Na,K-ATPase in terms of interactions with electrons, Bioelectromagnetics 26 (2005) 591–597.
[48] M. Blank, L. Soo, The threshold for alternating current inhibition of the Na,K-ATPase, Bioelectromagnetics 13 (1992) 329–333.
[49] M. Blank, L. Soo, The threshold for Na,K-ATPase stimulation by electromagnetic fields, Bioelectrochem Bioenerg 40 (1996) 63–65.
[50] C. Wan, T. Fiebig, S.O. Kelley, C.R. Treadway, J.K. Barton, Femtosecond dynamics of DNA-mediated electron transfer, Proc Nat Acad Sci U S A 96 (1999) 6014–6019.
[51] M. Blank, Electric stimulation of protein synthesis in muscle, Adv Chem 250 (1995) 143–153.
[52] M. Blank, R. Goodman, Initial interactions in electromagnetic field-induced biosynthesis, J Cell Physiol 199 (2004) 359–363.
[53] M. Blank, R. Goodman, A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation, J Cell Physiol 214 (2008) 20–26.
[54] C.J. Fecko, J.D. Eaves, J.J. Loparo, A. Tokmakoff, P.L. Geissler, Ultrafast hydrogen-bond dynamics in infrared spectroscopy of water, Science 301 (2003) 1698–1701.
[55] T.A. Litovitz, D. Kraus, J.M. Mullins, Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity, Biochem Biophys Res Comm 178 (1991) 862–865.
[56] T.A. Litovitz, D. Kraus, M. Penafiel, E.C. Elson, J.M. Mullins, The role of coherence time in the effect of microwaves on ornithine decarboxylase activity, Bioelectromagnetics 14 (1993) 395–403.
[57] J.M. Mullins, T.A. Litovitz, M. Penafiel, A. Desta, A. Krause, Intermittent noise affects EMF- induced ODC activity, Bioelectrochem Bioenerg 44 (1998) 237–242.
[58] J.M. Mullins, L.M. Penafiel, J. Juutilainen, T.A. Litovitz, Dose-response of electromagnetic field-induced ornithine decarboxylase activity, Bioelectrochem Bioenerg 48 (1999) 193–199.
[59] M. Blank, L. Soo, Electromagnetic acceleration of electron transfer reactions, J Cell Biochem 81 (2001) 278–283.
[60] A.L. Di Carlo, J.M. Mullins, T.A. Litovitz, Thresholds for EM field-induced hypoxia protection: evidence for a primary, electric field effect, Bioelectrochem 52 (2000) 9–16.
[61] H. Ahlbom, N. Day, M. Feychting, E. Roman, J. Skinner, J. Dockerty, M. Linet, M. McBride, J. Michaelis, J.H. Olsen, T. Tynes, P.K. Verkasalo, A pooled analysis of magneticfields and childhood leukemia, Brit J Cancer 83 (2000) 692–698.
[62] S. Greenland, A.R. Sheppard, W.T. Kaune, C. Poole, M.A.A. Kelsh, Pooled Analysis of Magnetic Fields, Wire Codes, and Childhood Leukemia, Epidemiology 11 (2000) 624–634.

References

[1] R. Goodman, C.A.L. Bassett, A. Henderson, Pulsing electromagnetic fields induce cellular transcription, Science 220 (1983) 1283–1285.

[2] R. Goodman, A. Henderson, Exposure of salivary gland cells to low frequency electromagnetic fields alters polypeptide synthesis, PNAS 85 (1988) 3928–3932.

[3] M. Blank, Protein and DNA interactions with electromagnetic fields, Electromagn Biol Med 28 (2008) 3–23.

[4] W.R. Adey, Tissue interactions with non-ionizing electromagnetic fields, Physiol Rev 61 (1981) 435–514.

[5] H. Lin, M. Blank, R. Goodman, A magnetic field responsive domain in the human HSP70 promoter, J Cell Biochem 75 (1999) 170–176.

[6] H. Lin, M. Blank, K. Rossol-Haseroth, R. Goodman, Regulating genes with electromagnetic response elements, J Cell Biochem 81 (2001) 143–148.

[7] REFLEX Project Report, 2004, A summary of the final report can be found at http://www.verum-foundation.de/www2004/html/pdf/euprojekte01/REFLEX_ProgressSummary_231104.pdf.

[8] H. Lai, N.P. Singh, Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells, Bioelectromagnetics 18 (1997) 156–165.

[9] Bioinitiative Report, edited by C. Sage, D. Carpenter, A Scientific Perspective on Health Risk of Electromagnetic Fields, published online 31 August 2007 http://www.bioinitiative.org/report/index.htm.

[10] M. Blank, O. Khorkova, R. Goodman, Changes in polypeptide distribution stimulated by different levels of EM and thermal stress, Bioelectrochem Bioenerg 33 (1994) 109–114.

[11] R. Goodman, M. Blank, H. Lin, O. Khorkova, L. Soo, D. Weisbrot, A.S. Henderson, Increased levels of hsp70 transcripts are induced when cells are exposed to low frequency electromagnetic fields, Bioelectrochem Bioenerg 33 (1994) 115–120.

[12] D. Kultz, Molecular, Evolutionary basis of the cellular stress response, Ann Rev Physiol 67 (2005) 225–257.

[13] R. Goodman, M. Blank, Magnetic field stress induces expression of hsp70, Cell Stress Chaperones 3 (1998) 79–88.

[14] F.M. Ritossa, A new puffing pattern induced by a temperature shock and DNP in *Drosophila*, Experientia Basel 18 (1962) 571–573.

[15] D.I. de Pomerai, C. Daniells, H. David, J. Allan, I. Duce, M. Mutwakil, D. Thomas, P. Sewell, J. Tattersall, D. Jones, Non-thermal heat-shock response to microwaves, Nature 6785 (2000) 417–418.

[16] J. Czyz, K. Guan, Q. Zeng, T. Nikolova, A. Meister, F. Schönborn, I. Schuderer, N. Kuster, A.M. Wobus, High frequency electromagnetic fields (GSM signals) affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells, Bioelectromagnetics 25 (2004) 296–307.

[17] T. Taira, Y. Negishi, F. Kihara, S.M.M. Iguchi-Ariga, H. Ariga, H. c-myc protein complex binds to two sites in human hsp70 promoter region, Biochim Biophys Acta 1130 (1992) 166–174.

[18] J. Topol, D.M. Ruden, C.S. Parker, Sequences required for in vitro transcriptional activation of a drosophila hsp70 gene, Cell 42 (1985) 527–537.

[19] A. Albertini, B. Zucchini, G. Noeru, R. Cadossi, C.P. Napoleone, A. Pierangeli, Protective effect of low frequency low energy policy electromagnetic fields on acute experimental myocardial infarcts in rats, Bioelectromagnetics 20 (1999) 372–377.

[20] A. Di Carlo, J.M. Farrell, T. Litovitz, A simple experiment to study electromagnetic field effects: protection induced by short-term exposures to 60 Hz magnetic fields, Bioelectromagnetics 19 (1998) 498–500.

[21] J.M. Shallom, A.L. DiCarlo, D. Ko, L.M. Penafiel, A. Nakai, Microwave exposure induces hsp70 and confers protection against hypoxia in chick embryos, J Cell Biochem 86 (2002) 490–496.

[22] I. George, M. Geddis, Z. Lill, H. Lin, T. Gomez, M. Blank, M. Oz, R. Goodman, Myocardial function improved by electromagnetic fields induction of stress protein hsp70, J Cellular Physiol 216 (2008) 816–823, published online: doi:10.1002/jcp.21461.

[23] J. Friedman, S. Kraus, Y. Hauptman, Y. Schiff, R. Seger, Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies, Biochem J 405 (2007) 559–568.

[24] M. Jin, M. Blank, R. Goodman, ERK1/2 phosphorylation, induced by electromagnetic fields, diminishes during neoplastic transformation, J Cell Biochem 78 (2000) 371–379.

[25] D. Leszczynski, S. Joenvaara, J. Reivinen, R. Kuokka, Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer-and blood-brain barrier-related effects, Differentiation 70 (2002) 120–129.

[26] M. Simko, Induction of cell activation processes by low frequency electromagnetic fields, ScientificWorldJournal 4 (Suppl. 2) (2004) 4–22.

[27] R. Marais, J. Wynne, R. Treisman, The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain, Cell 73 (1993) 381–393.

[28] R. Janecht, W.H. Ernst, V. Pigoud, A. Nordheim, Activation of TCF Elk-1 by MAP kinases, EMBO J 12 (1993) 5097–5104.

[29] H. Gille, M. Kortenjann, O. Thomae, C. Moomaw, C. Slaughter, M.H. Cobb, P.E. Shaw, ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation, EMBO J 14 (1995) 951–962.

[30] V. Sgambato, P. Vanhoutte, C. Pages, M. Rogard, L. Hipskind, M.J. Besson, J. Caboche, In vivo expression and regulation of Elk-1, a target of the extracellular-regulated kinase signaling pathway, in the rat brain, J. Neurosci 18 (1998) 214–226.

[31] D. Leszczynski, R. Nylund, S. Joevaara, J. Reivinen, Applicability of discovery science approach to determine biological effects of mobile phone radiation, Proteonomics 4 (2004) 426–431.

[32] G.J. Hook, P. Zhang, I. Lagroye, L.E. Li, R. Higashikubo, E.G. Moros, W.L. Starube, W.F. Pickard, J.D. Baty, J.L. Roti-Roti, Measurement of DNA damage and apoptosis in Molt-4 cells after in vitro exposure to radio frequency radiation, Radiat Res 161 (2004) 193–200.

[33] D. Weisbrot, H. Lin, L. Ye, M. Blank, R. Goodman, Effects of mobile phone radiation on growth and development in *Drosophila melanogaster*, J Cell Biochem 89 (2003) 48–55.

[34] S. Rao, A.S. Henderson, Regulation of c-fos is affected by electromagnetic fields, J Cell Biochem 63 (1996) 358–365.

[35] D.L. Johnson, S.A.S. Johnson, RNA metabolism and oncogenesis, Science 320 (2008) 461–462.

[36] I.A. Cotgreave, Biological stress responses to radio frequency electromagnetic radiation: are mobile phones really so (heat) shocking? Arch Biochem Biophys 435 (2005) 227–240.

[37] M. Jin, H. Lin, L. Han, M. Opler, S. Maurer, M. Blank, R. Goodman, Biological and technical variables in myc expression in HL60 cells exposed to 60 Hz electromagnetic fields, Bioelectrochem Bioenerg 44 (1997) 111–120.

[38] R.P. Liburdy, T.R. Sloma, R. Sokolic, P. Yaswen, ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation, J Pineal Res 14 (1993) 89–97.

[39] H. Lin, M. Head, M. Blank, L. Han, M. Jin, R. Goodman, Myc-mediated transactivation of HSP70 expression following exposure to magnetic fields, J Cell Biochem 69 (1998) 181–188.

[40] S. Ivancsits, A. Pilger, F. Diem, O. Jahn, H. Rudiger, Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields, Mutation Res 583 (2005) 184–188.

[41] M. Lantow, M. Lupke, J. Frahm, M.O. Mattsson, N. Kuster, M. Simko, ROS release and Hsp70 expression after exposure to 1,800 MHz radiofrequency electromagnetic fields in primary human monocytes and lymphocytes, Radiat Environ Biophys 45 (2006) 55–62.

[42] M. Simko, C. Hartwig, M. Lantow, M. Lupke, M.O. Mattsson, Q. Rahman, J. Rollwitz, Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultrafine particles in human Mono Mac 6 cells, Toxicol Lett 161 (2006) 73–82.

ELSEVIER

Pathophysiology 16 (2009) 79–88

ISP
PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Electromagnetic fields and DNA damage

J.L. Phillips [a,*], N.P. Singh [b], H. Lai [b]

[a] Department of Chemistry, University of Colorado at Colorado Springs, Colorado Springs, CO 80918, USA
[b] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA

Received 24 October 2008; received in revised form 16 November 2008; accepted 16 November 2008

Abstract

A major concern of the adverse effects of exposure to non-ionizing electromagnetic field (EMF) is cancer induction. Since the majority of cancers are initiated by damage to a cell's genome, studies have been carried out to investigate the effects of electromagnetic fields on DNA and chromosomal structure. Additionally, DNA damage can lead to changes in cellular functions and cell death. Single cell gel electrophoresis, also known as the 'comet assay', has been widely used in EMF research to determine DNA damage, reflected as single-strand breaks, double-strand breaks, and crosslinks. Studies have also been carried out to investigate chromosomal conformational changes and micronucleus formation in cells after exposure to EMF. This review describes the comet assay and its utility to qualitatively and quantitatively assess DNA damage, reviews studies that have investigated DNA strand breaks and other changes in DNA structure, and then discusses important lessons learned from our work in this area.

Keywords: Electromagnetic field; DNA damage; Comet assay; Radiofrequency radiation; Cellular telephone

1. The comet assay for measurement of DNA strand breaks

DNA is continuously damaged by endogenous and exogenous factors and then repaired by DNA repair enzymes. Any imbalance in damage and repair and mistakes in repair result in accumulation of DNA damage. Eventually, this will lead to cell death, aging, or cancer. There are several types of DNA lesions. The common ones that can be detected easily are DNA strand breaks and DNA crosslinks. Strand breaks in DNA are produced by endogenous factors, such as free radicals generated by mitochondrial respiration and metabolism, and by exogenous agents, including UV, ionizing and non-ionizing radiation, and chemicals.

There are two types of DNA strand breaks: single- and double-strand breaks. DNA single-strand breaks include frank breaks and alkali labile sites, such as base modification, deamination, depurination, and alkylation. These are the most commonly assessed lesions of DNA. DNA double-strand breaks are very critical for cells and usually they are lethal. DNA strand breaks have been correlated with cell death [1–5], aging [6–8] and cancer [9–13].

Several techniques have been developed to analyze single- and double-strand breaks. Most commonly used is microgel electrophoresis, also called the 'comet assay' or 'single cell gel electrophoresis'. This technique involves mixing cells with agarose, making microgels on a microscope slide, lysing cells in the microgels with salts and detergents, removing proteins from DNA by using proteinase K, unwinding/equilibrating and electrophoresing DNA (under highly alkaline condition for assessment of single-strand breaks or under neutral condition for assessment of DNA double-strand breaks), fixing the DNA, visualizing the DNA with a fluorescent dye, and then analyzing migration patterns of DNA from individual cells with an image analysis system.

The comet assay is a very sensitive method of detecting single- and double-strand breaks if specific criteria are met. Critical criteria include the following. Cells from tissue culture or laboratory animals should be handled with care to minimize DNA damage, for instance, by avoiding light and high temperature. When working with animals exposed to EMF *in vivo*, it is better to anesthetize the animals with CO_2 before harvesting tissues for assay. Antioxidants

* Corresponding author.
E-mail address: jphillip@mail.uccs.edu (J.L. Phillips).

0928-4680/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2008.11.005

such as albumin and sucrose, or spin-trap molecules such as α-phenyl-*tert*-butyl nitrone (PBN), should be added during dispersion of tissues into single cells. Cells should be lysed at 0–4 °C to minimize DNA damage by endonucleases. Additionally, antioxidants such as tris and glutathione, and chelators such as EDTA, should be used in the lysing solution. High concentrations of dimethylsulfoxide (DMSO) should be avoided due to its chromatin condensing effect. Treatment with proteinase K (PK; lyophilized DNAse-free proteinase-K from Amresco is ideal) at a concentration of 0.5–1 mg/ml (depending upon cell type and number of cells in the microgel) should be used for 1–2 h at 37 °C to reveal all possible strand breaks which otherwise may go undetected due to DNA–protein crosslinks. Longer times in PK will lead to loss of smaller pieces of DNA by diffusion. Glass slides should be chosen based on which high resolution agarose (3:1 high resolution agarose from Amresco is ideal) will stick well to the slide and on the ability of the specimen to be visualized without excessive fluorescence background. Choice of an electrophoresis unit is important to minimize slide-to-slide variation in DNA migration pattern. A unit with uniform electric field and buffer recirculation should be used. Electrophoresis buffers should have antioxidants and chelators such as DMSO and EDTA. DNA diffusion should be minimized during the neutralization step by rapidly precipitating the DNA. Staining should employ a sensitive fluorescent dye, such as the intercalating fluorescent labeling dye YOYO-1. A cell-selection criteria for analysis should be set before the experiment, such as not analyzing cells with too much damage, although, the number of such cells should be recorded.

There are different versions of the comet assay that have been modified to meet the needs of specific applications and to improve sensitivity. Using the most basic form of the assay, one should be able to detect DNA strand breaks in human lymphocytes that were induced by 5 rad of gamma-ray [14,15].

2. Radiofrequency radiation (RFR) and DNA damage

In a series of publications, Lai and Singh [16–19] reported increases in single- and double-strand DNA breaks, as measured by the comet assay, in brain cells of rats exposed for 2 h to a 2450-MHz RFR at whole body specific absorption rate (SAR) between 0.6 and 1.2 W/kg. The effects were blocked by antioxidants, which suggested involvement of free radicals. At the same time, Sarkar et al. [20] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150, and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals. Their data also suggested breakage of DNA strands after RFR exposure. Phillips et al. [21] were the first to study the effects of two forms of cell cellular phone signals, known as TDMA and iDEN, on DNA damage in Molt-4 human lymphoblastoid cells using the comet assay. These cells were exposed to relatively low intensities of the fields (2.4–26 μW/g) for 2–21 h. They reported both increased and decreased DNA damage, depending on the type of signal studied, as well as the intensity and duration of exposure. They speculated that the fields may affect DNA repair in cells. Subsequently, different groups of researchers have also reported DNA damage in various types of cells after exposure to cell phone frequency fields. Diem et al. [22] exposed human fibroblasts and rat granulosa cells to cell phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; for 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h of exposure to different cell phone modulations in both cell types. The intermittent exposure schedule caused a significantly stronger effect than continuous exposure. Gandhi and Anita [23] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users. Markova et al. [24] reported that GSM signals affected chromatin conformation and γ-H2AX foci that co-localized in distinct foci with DNA double-strand breaks in human lymphocytes. The effect was found to be dependent on carrier frequency. Nikolova et al. [25] reported a low and transient increase in DNA double-strand breaks in mouse embryonic stem cells after acute exposure to a 1.7-GHz field. Lixia et al. [26] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 h of exposure to a 1.8-GHz field at 3 W/kg. Sun et al. [27] reported an increase in DNA single-strand breaks in human lens epithelial cells after 2 h of exposure to a 1.8-GHz field at SARs of 3 and 4 W/kg. DNA damage caused by the field at 4 W/kg was irreversible. Zhang et al. [28] reported that an 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 h of exposure. Aitken et al. [29] exposed mice to a 900-MHz RFR at a SAR of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as by alkaline and pulsed-field gel electrophoresis. Gel electrophoresis revealed no significant change in single- or double-strand breaks in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome and the nuclear β-globin locus. Changes in sperm cell genome after exposure to 2450-MHz microwaves have also been reported previously by Sarkar et al. [20]. Related to this are several publications that have reported decreased motility and changes in morphology in isolated sperm cells exposed to cell phone radiation [30], sperm cells from animals exposed to cell phone radiation [31], and cell phone users [32–34]. Some of these *in vivo* effects could be caused by hormonal changes [35,36].

There also are studies reporting no significant effect of cell phone RFR exposure on DNA damage. After RFR-induced DNA damage was reported by Lai and Singh [16] using 2450-MHz microwaves and after the report of Phillips et al. [21] on cell phone radiation was published, Motorola funded a series of studies by Roti Roti and colleagues [37] at

Washington University to investigate DNA strand breaks in cells and animals exposed to RFR. None of the studies reported by this group found significant effects of RFR exposure on DNA damage [38–40]. However, a different version of the comet assay was used in these studies. More recently, four additional studies from the Roti-Roti laboratories also reported no significant effects on DNA damage in cells exposed to RFR. Li et al. [41] reported no significant change in DNA strand breaks in murine C3H10T1/2 fibroblasts after 2 h of exposure to 847.74- and 835.02-MHz fields at 3–5 W/kg. Hook et al. [42] showed that a 24-h exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA-modulated RFR did not significantly alter the level of DNA damage. Lagroye et al. [43,44] also reported no significant change in DNA strand breaks, protein–DNA crosslinks, and DNA–DNA crosslinks in cells exposed to 2450-MHz RFR.

From other laboratories, Vijayalaxmi et al. [45] reported no increase in DNA stand breaks in human lymphocytes exposed *in vitro* to 2450-MHz RFR at 2.135 W/kg for 2 h. Tice et al. [46] measured DNA single-strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed for 3 or 24 h at average SARs of 1.0–10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes. McNamee et al. [47–49] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 h to a 1.9-GHz field at SAR up to 10 W/kg. Zeni et al. [50] reported that a 2-h exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes. Sakuma et al. [51] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to cell phone radiation for 2 and 24 h. No significant changes in DNA strand breaks were observed up to a SAR of 800 mW/kg. Stronati et al. [52] showed that 24 h of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells. Verschaeve et al. [53] reported that long-term exposure (2 h/day, 5 days/week for 2 years) of rats to 900-MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

3. Extremely low frequency electromagnetic fields (ELF EMF) and DNA damage

To complete the picture, a few words on the effects of ELF EMF are required, since cell phones also emit these fields and they are another common form of non-ionizing EMF in our environment. Quite a number of studies have indicated that exposure to ELF EMF could lead to DNA damage [54–69]. In addition, two studies [70,71] have reported effects of ELF fields on DNA repair mechanisms. Free radicals and interaction with transitional metals (e.g., iron) [60,62,63,69] have also been implicated to play a role in the genotoxic effects observed after exposure to these fields.

4. Some considerations on the effects of EMF on DNA

From this brief literature survey, no consistent pattern of RFR exposure inducing changes in or damage to DNA in cells and organisms emerges. However, one can conclude that under certain conditions of exposure, RFR is genotoxic. Data available are mainly applicable only to radiation exposure that would be typical during cell phone use. Other than the study of Phillips et al. [21], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

Differences in experimental outcomes are expected since many factors could influence the outcome of experiments in EMF research. Any effect of EMF has to depend on the energy absorbed by a biological organism and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these factors have been discussed in a talk given by one us (HL) in Vienna, Austria in 1998 [72].

Radiation from cell phone transmission has very complex patterns, and signals vary with the type of transmission. Moreover, the technology is constantly changing. Research results from one types of transmission pattern may not be applicable to other types. Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [21], which demonstrated that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks after exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used poor exposure procedures with very limited documentation of exposure parameters, e.g., using an actual cell phone to expose cells and animals, thus rendering the data from these experiments as questionable.

Another source of influence on experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [73] and organisms [74,75] may not all respond similarly to EMF.

Comment about the comet assay also is required, since it was used in many of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay could be misleading. Another concern is that most of the comet assay studies were carried out by experimenters who had no prior experience with this technique and mistakes

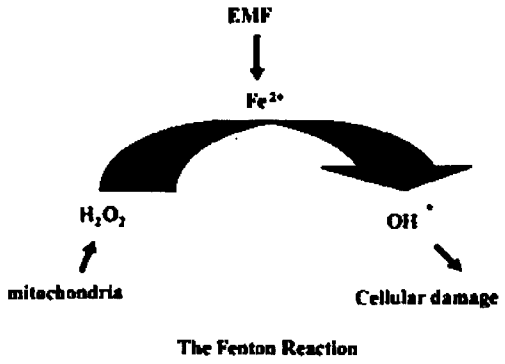

Fig. 1. A representation of the Fenton reaction and its role as a mediator in EMF-induced bioeffects.

were made. For example, in the study by Lagroye et al. [43] to investigate the effect of PK digestion on DNA migration after RFR exposure, PK was added to a lysing solution containing the detergent Triton X-100, which would inactivate the enzyme. Our experience indicates that the comet assay is a very sensitive and requires great care to perform. Thus, different detection sensitivities could result in different laboratories, even if the same procedures are followed. One way to solve this problem of experimental variation is for each research team to report the sensitivity of their comet assay, e.g., the threshold of detecting strand breaks in human lymphocytes exposed to X-rays. This information has generally not been provided for EMF-genotoxicity studies. Interestingly, when such information was provided, a large range of sensitivities have been reported. Malyapa et al. [40] reported a detection level of 0.6 cGy of gamma radiation in human lymphocytes, whereas McNamee et al. [76] reported 10–50 cGy of X-irradiation in lymphocytes, which is much higher than the generally acceptable detection level of the comet assay [15].

A drawback in the interpretation and understanding of experimental data from bioelectromagnetics research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which EMF produces changes in DNA is unknown. Since the energy level associated with EMF exposure is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced biochemical changes in cells.

One possibility is that DNA is damaged by free radicals that are formed inside cells. Free radicals affect cells by damaging macromolecules, such as DNA, protein, and membrane lipids. Several reports have indicated that EMF enhances free radical activity in cells [18,19,61,62,77,78], particularly via the Fenton reaction [62]. The Fenton reaction is a process catalyzed by iron in which hydrogen peroxide, a product of oxidative respiration in the mitochondria, is converted into hydroxyl free radicals, which are very potent and cytotoxic molecules (Fig. 1).

It is interesting that ELF EMF has also been shown to cause DNA damage. Furthermore, free radicals have been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR. Effects via the Fenton reaction predict how a cell would respond to EMF. For instance:

(1) Cells that are metabolically active would be more susceptible to EMF, because more hydrogen peroxide is generated by mitochondria to fuel the reaction.
(2) Cells that have high level of intracellular free iron would be more vulnerable to EMF. Cancer cells and cells undergoing abnormal proliferation have higher concentrations of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF. Consequently, this suggests that EMF could potentially be used for the treatment of cancer and hyperplastic diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells. One consequence of this consideration is that epidemiological studies of cancer incidence in cell phone users may not show a risk at all or even a protection effect.
(3) Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high levels of iron. Special molecular pumps are present on nerve cell nuclear membranes to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capacity for DNA repair, and DNA breaks could easily accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues, particularly in the brain. These particles could enhance free radical activity in cells and thus increase the cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, the more likely consequences of DNA damage in nerve cells include changes in cellular functions and in cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double-strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cell, the glial cell, can become cancerous as a result of DNA damage. The question is whether the damaged cells

would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors—an increase, decrease, or no significant change in cancer risk could result from EMF exposure.

(4) On the other hand, cells with high amounts of antioxidants and antioxidative enzymes would be less susceptible to EMF. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

Additionally, the work of Blank and Soo [79] and Blank and Goodman [80] support the possibility that EMF exposure at low levels has a direct effect on electron transfer processes. Although the authors do not discuss their work in the context of EMF-induced DNA damage, the possibility exists that EMF exposure could produce oxidative damage to DNA.

5. Lessons learned

Whether or not EMF causes biological effects, let alone effects that are detrimental to human health and development, is a contentious issue. The literature in this area abounds with apparently contradictory studies, and as presented in this review, the literature specific to the effects of RFR exposure on DNA damage and repair in various biological systems is no exception. As a consequence of this controversy, there are several key issues that must be addressed—contrary data, weight of evidence, and data interpretation consistent with known science.

Consider that EMF does not share the familiar and comforting physical properties of chemical agents. EMF cannot be seen, tasted, smelled, or felt (except at high intensities). It is relevant, therefore, to ask, in what ways do scientists respond to data, especially if that data are contrary to their scientific beliefs or inconsistent with long-held hypotheses? Often such data are ignored, simply because it contradict what is accepted as conventional wisdom. Careful evaluation and interpretation of data may be difficult, because technologies used to expose biological systems to EMF and methodologies used to assess dosimetry generally are outside the experience of most biomedical scientists. Additionally, it is often difficult to assess differences in methodologies between studies, one or more of which were intended to replicate an original investigation. For instance, Malyapa et al. [40] reported what they claimed to be a replication of the work of Lai and Singh [16]. There were, however, significant differences in the comet analyses used by each group. Lai and Singh precipitated DNA in agarose so that low levels of DNA damage could be detected. Malyapa et al. did not. Lai and Singh treated their samples with PK to digest proteins bound to DNA, thus allowing DNA to move toward the positive pole during electrophoresis (unlike DNA, most proteins are negatively charged, and if they are not removed they will drag the DNA toward the negative pole). The Malyapa et al. study did not use PK. There were other methodological differences as well. Such is also the case in the study of Hook et al. [42], which attempted to replicate the work of Phillips et al. [21]. The latter group used a PK treatment in their comet assay, while the former group did not.

While credibility is enhanced when one can relate data to personal knowledge and scientific beliefs, it has not yet been determined how RFR couples with biological systems or by what mechanisms effects are produced. Even carefully designed and well executed RFR exposure studies may be summarily dismissed as methodologically unsound, or the data may be interpreted as invalid because of inconsistencies with what one believes to be correct. The quintessential example is the belief that exposure to RFR can produce no effects that are not related to the ability of RFR to produce heat, that is, to raise the temperature of biological systems [81,82]. Nonetheless, there are many examples of biological effects resulting from low-level (athermal) RFR exposure [83,84]. Consider here the work of Mashevich et al. [85]. This group exposed human peripheral blood lymphocytes to an 830-MHz signal for 72 h and at different average SARs (SAR, 1.6–8.8 W/kg). Temperatures ranged from 34.5 to 38.5 °C. This group observed an increase in chromosome 17 aneuploidy that varied linearly with SAR. Temperature elevation alone in the range of 34.5–38.5 °C did not produce this genotoxic effect, although significant aneuploidy was observed at higher temperatures of 40–41 °C. The authors conclude that the genotoxic effect of the radiofrequency signal used is elicited through a non-thermal pathway.

Also consider one aspect of the work of Phillips et al. [21]. In that study, DNA damage was found to vary in direction; that is, under some conditions of signal characteristics, signal intensity, and time of exposure, DNA damage increased as compared with concurrent unexposed controls, while under other conditions DNA damage decreased as compared with controls. The dual nature of Phillips et al.'s [21] results will be discussed later. For now consider the relationship of these results to other investigations. Adey et al. [86] performed an *in vivo* study to determine if rats treated *in utero* with the carcinogen ethylnitrosourea (ENU) and exposed to an 836.55-MHz field with North American Digital Cellular modulation (referred to as a TDMA field) would develop increased numbers of central system tumors. This group reported that rather than seeing an increase in tumor incidence in RFR-exposed rats, there was instead a decrease in tumor incidence. Moreover, rats that received no ENU but which were exposed to the TDMA signal also showed a decrease in the number of spontaneous tumors as compared

with animals exposed to neither ENU nor the TDMA signal. This group postulated that their results may be mechanistically similar to the work of another group. Stammberger et al. [87] had previously reported that rats treated *in utero* with ENU and then exposed to low doses of X-irradiation exhibited significantly reduced incidences of brain tumors in adult life. Stammberger and colleagues [87] hypothesized that low-level X-irradiation produced DNA damage that then induced the repair enzyme O^6-alkylguanine-DNA alkyltransferase (AT). Numerous groups have since reported that X-irradiation does indeed induce AT activity (e.g., [88,89]). In this context, it is significant that Phillips et al. [21] found that cells exposed *in vitro* to a TDMA signal identical to that used in the study of Adey et al. [86] produced a decrease in DNA damage under specific conditions of intensity and time of exposure (lower intensity, longer time; higher intensity, shorter time). These results raise the intriguing possibility that the decrease in tumor incidence in the study of Adey et al. [86] and the decrease in DNA damage in the study of Phillips et al. [21] both may have been the result of induction of AT activity resulting from DNA damage produced by exposure to the TDMA signal. This remains to be investigated.

Because the issue of RFR-induced bioeffects is contentious, and because the issue is tried in courtrooms and various public forums, a term heard frequently is weight of evidence. This term generally is used to describe a method by which all scientific evidence related to a causal hypothesis is considered and evaluated. This process is used extensively in matters of regulation, policy, and the law, and it provides a means of weighing results across different modalities of evidence. When considering the effects of RFR exposure on DNA damage and repair, modalities of evidence include studies of cells and tissues from laboratory animals exposed *in vivo* to RFR, studies of cells from humans exposed to RFR *in vivo*, and studies of cells exposed *in vitro* to RFR. While weight of evidence is gaining favor with regulators [90], its application by scientists to decide matters of science is often of questionable value. One of the reasons for this is that there generally is no discussion or characterization of what weight of evidence actually means in the context in which it is used. Additionally, the distinction between weight of evidence and strength of evidence often is lacking or not defined, and differences in methodologies between investigators are not considered. Consequently, weight of evidence generally amounts to what Krimsky [90] refers to as a "seat-of-the-pants qualitative assessment." Krimsky points out that according to this view, weight of evidence is "a vague term that scientists use when they apply implicit, qualitative, and/or subjective criteria to evaluate a body of evidence." Such is the case in the reviews by Juutilainen and Lang [91] and Verschaeve and Maes [92]. There is little emphasis on a critical analysis of similarities and differences in biological systems used, exposure regimens, data produced, and investigator's interpretations and conclusions. Rather, there is greater emphasis on the number of publications either finding or not finding an effect of RFR exposure on some endpoint. To some investigators, weight of evidence does indeed refer to the balance (or imbalance) between the number of studies producing apparently opposing results, without regard to critical experimental variables. While understanding the role these variables play in determining experimental outcome could provide remarkable insights into defining mechanisms by which RFR produced biological effects, few seem interested in or willing to delve deeply into the science.

A final lesson can be derived from a statement made by Gos et al. [93] referring to the work of Phillips et al. [21]. Gos and colleagues state, "The results in the latter study (Phillips et al., 1998) are puzzling and difficult to interpret, as no consistent increase or decrease in signal in the comet assay at various SARs or times of exposure was identified." This statement is pointed out because studies of the biological effects of exposure to electromagnetic fields at any frequency are often viewed as outside of or distinct from what many refer to as *mainstream science*. However, what has been perceived as an inconsistent effect is indeed consistent with the observations of bimodal effects reported in hundreds of peer-reviewed publications. These bimodal effects may be dependent on concentration of an agent, time of incubation with an agent, or some other parameter relating to the state of the system under investigation. For instance, treatment of B cells for a short time (30 min) with the protein kinase C activator phorbol 12,13-dibutyrate increased proliferative responses to anti-immunoglobulin antibody, whereas treatment for a longer period of time ($\geq$3 h) suppressed proliferation [94]. In a study of κ-opioid agonists on locomotor activity in mice, Kuzmin et al. [95] reported that higher, analgesic doses of κ-agonists reduced rearing, motility, and locomotion in non-habituated mice. In contrast, lower, subanalgesic doses increased motor activity in a time-dependent manner. Dierov et al. [96] observed a bimodal effect of all-trans-retinoic acid (RA) on cell cycle progression in lymphoid cells that was temporally related to the length of exposure to RA. A final example is found in the work of Rosenstein et al. [97]. This group found that the activity of melatonin on depolarization-induced calcium influx by hypothalamic synaptosomes from rats sacrificed late evening (2000 h) depended on melatonin preincubation time. A short preincubation time (10 min) stimulated uptake, while a longer preincubation (30 min) inhibited calcium uptake. These effects were also dependent on the time of day when the rats were sacrificed. Effects were maximal at 2000 h, minimal at 2400 h, and intermediate at 400 h. At 1000 h, only inhibitory effects of melatonin on calcium uptake were observed. These examples point out that what appears to be inconsistency may instead be real events related to and determined by the agents involved and the state of the biological system under investigation. The results of Phillips et al. [21] may be the result of signal modulation, signal intensity, time of exposure, or state of the cells. The results may indicate a bimodal effect, or they may, as the investigators suggest, represent time- and signal-dependant changes in the balance between damage and repair because of direct or indirect effects of RFR exposure on repair mechanisms.

6. Summary

Exposure of laboratory animals *in vivo* and of cultured cells *in vitro* to various radiofrequency signals has produced changes in DNA damage in some investigations and not in others. That many of the studies on both sides of this issue have been done well is encouraging from a scientific perspective. RFR exposure does indeed appear to affect DNA damage and repair, and the total body of available data contains clues as to conditions producing effects and methodologies to detect them. This view is in contrast to that of those who believe that studies unable to replicate the work of others are more credible than the original studies, that studies showing no effects cancel studies showing an effect, or that studies showing effects are not credible simply because we do not understand how those effects might occur. Some may be tempted to apply incorrectly the teachings of Sir Karl Popper, one of the great science philosophers of the 20th century. Popper proposed that many examples may lend support to an hypothesis, while only one negative instance is required to refute it [98]. While this holds most strongly for logical subjects, such as mathematics, it does not hold well for more complex biological phenomena that are influenced by stochastic factors. Each study to investigate RFR-induced DNA damage must be evaluated on its own merits, and then studies that both show effects and do not show effects must be carefully evaluated to define the relationship of experimental variables to experimental outcomes and to assess the value of experimental methodologies to detect and measure these outcomes (see Section 2).

The lack of a causal or proven mechanism(s) to explain RFR-induced effects on DNA damage and repair does not decrease the credibility of studies in the scientific literature that report effects of RFR exposure, because there are several plausible mechanisms of action that can account for the observed effects. The relationship between cigarette smoking and lung cancer was accepted long before a mechanism was established. This, however, occurred on the strength of epidemiologic data [99]. Fortunately, relevant epidemiologic data relating long-term cell phone use (>10 years) to central nervous system tumors are beginning to appear [84,100–102], and these data point to an increased risk of acoustic neuroma, glioma and parotid gland tumors.

One plausible mechanism for RFR-induced DNA damage is free radical damage. After finding that two free radical scavengers (melatonin and N-tert-butyl-α-phenylnitrone) prevent RFR-induced DNA damage in rat brain cells, Lai and Singh [62] hypothesized that this damage resulted from free radical generation. Subsequently, other reports appeared that also suggested free radical formation as a result of RFR exposure [103–105]. Additionally, some investigators have reported that non-thermal exposure to RFR alters protein structure and function [106–109]. Scientists are familiar with molecules interacting with proteins through lock-and-key or induced-fit mechanisms. It is accepted that such interactions provide energy to change protein conformation and protein function. Indeed, discussions of these principles are presented in introductory biology and biochemistry courses. Perhaps then it is possible that RFR exposure, in a manner similar to that of chemical agents, provides sufficient energy to alter the structure of proteins involved in DNA repair mechanisms to the extent that their function also is changed. This has not yet been investigated.

When scientists maintain their beliefs in the face of contrary data, two diametrically opposed situations may result. On the one hand, data are seen as either right or wrong and there is no discussion to resolve disparities. On the other hand, and as Francis Crick [110] has pointed out, scientists who hold theoretically opposed positions may engage in fruitful debate to enhance understanding of underlying principles and advance science in general. While the latter certainly is preferable, there are external factors involving economics and politics that keep this from happening. It is time to acknowledge this and embark on the path of fruitful discussion. Great scientific discoveries await.

Acknowledgment

We thank Khushbu Komal and Ji-Sun Park for assistance in the preparation of the manuscript.

References

[1] J.F. Ward, The yield of DNA double strand breaks produced intracellularly by ionizing radiation: a review, Int. J. Radiat. Biol. 57 (1990) 1141–1150.
[2] P.R. Walker, C. Smith, T. Youdale, J. Leblanc, J.F. Whitfield, M. Sikorska, Topoisomerase II-reactive chemotherapeutic drugs induce apoptosis in thymocytes, Cancer Res. 51 (1991) 1078–1085.
[3] Y. Onishi, Y. Azuma, Y. Sato, Y. Mizuno, T. Tadakuma, H. Kizaki, Topoisomerase inhibitors induce apoptosis in thymocytes, Biochem. Biophys. Acta 1175 (1993) 147–154.
[4] P. Prigent, C. Blanpied, J. Aten, F. Hirsch, A safe and rapid method for analyzing apoptosis-induced fragmentation of DNA extracted from tissues or cultured cells, J. Immunol. Methods 160 (1993) 139–140.
[5] K. Schindowski, S. Leutner, W.E. Muller, A. Eckert, Age related changes of apoptotic cell death in human lymphocytes, Neurobiol. Aging 21 (2000) 661–670.
[6] R.W. Hart, R.B. Setlow, Correlation between deoxyribonucleic acid excision repair and life span in a number of mammalian species, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 2169–2173.
[7] M.R. Lieber, Pathological and physiological double-strand breaks: roles in cancer, aging, and theimmune system, Am. J. Pathol. 153 (1998) 1323–1332.
[8] H. Li, J.R. Mitchell, P. Hasty, DNA double-strand breaks: a potential causative factor for mammalian aging, Mech. Aging Dev. 129 (2008) 416–424.
[9] R.R. R.R.Tice, Aging and DNA repair capability, in: E.L. Schneider (Ed.), The Genetics of Aging, Plenum Press, New York, 1978, pp. 53–89.
[10] P.A. Cerutti, Peroxidant states and tumor promotion, Science 227 (1985) 375–381.
[11] B.N. Ames, Endogenous DNA damage as related to cancer and aging, Mutat. Res. 214 (1989) 41–46.

[12] B.N. Ames, Endogenous oxidative DNA damage, aging, and cancer, Free Radical Res. Commun. 7 (1989) 121–128.
[13] T. Helleday, J. Loc, D.C. van Gentd, B.P. Engelward, DNA double-strand break repair: from mechanistic understanding to cancer treatment, DNA Repair 6 (2007) 923–935.
[14] R.B. Jarvis, J.F. Knowles, DNA damage in Zebrafish larvae induced by exposure to low-dose rate gamma-radiation: detection by the alkaline comet assay, Mutat. Res. 541 (2003) 63–69.
[15] R.R. Tice, G.H. Strauss, The single cell gel electrophoresis/comet assay: a potential tool for detecting radiation-induced DNA damage in humans, Stem Cells. Suppl. 1 (1995) 207–214.
[16] H. Lai, N.P. Singh, Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells, Bioelectromagnetics 16 (1995) 207–210.
[17] H. Lai, N.P. Singh, Single and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation, Int. J. Radiat. Biol. 69 (1996) 513–521.
[18] H. Lai, N.P. Singh, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells, Bioelectromagnetics 18 (1997) 446–454.
[19] H. Lai, N.P. Singh, Effects of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells, Electromag. Biol. Med. 24 (2005) 23–29.
[20] S. Sarkar, S. Ali, J. Behari, Effect of low power microwave on the mouse genome: a direct DNA analysis, Mutat. Res. 320 (1994) 141–147.
[21] J.L. Phillips, O. Ivaschuk, T. Ishida-Jones, R.A. Jones, M. Campbell-Beachler, W. Haggren, DNA damage in Molt-4 T- lymphoblastoid cells exposed to cellular telephone radiofrequency fields *in vitro*, Bioelectrochem. Bioenerg. 45 (1998) 103–110.
[22] E. Diem, C. Schwarz, F. Adlkofer, O. Jahn, H. Rudiger, Non-thermal DNA breakage by mobile-phone radiation (1800-MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro, Mutat. Res. 583 (2005) 178–183.
[23] G. Gandhi, Anita, Genetic damage in mobile phone users: some preliminary findings, Indian J. Hum. Genet. 11 (2005) 99–104.
[24] E. Markova, L. Hillert, L. Malmgren, B.R. Persson, I.Y. Belyaev, Microwaves from GSM mobile telephones affect 53BP1 and gamma-H2AX foci in human lymphocytes from hypersensitive and healthy persons, Environ. Health Perspect. 113 (2005) 1172–1177.
[25] T. Nikolova, J. Czyz, A. Rolletschek, P. Blyszczuk, J. Fuchs, G. Jovtchev, J. Schuderer, N. Kuster, A.M. Wobus, Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells, FASEB J. 19 (2005) 1686–1688.
[26] S. Lixia, K. Yao, W. Kaijun, L. Deqiang, H. Huajun, G. Xiangwei, W. Baohong, Z. Wei, L. Jianling, W. Wei, Effects of 1.8-GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells, Mutat. Res. 602 (2006) 135–142.
[27] L.X. Sun, K. Yao, J.L. He, D.Q. Lu, K.J. Wang, H.W. Li, Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro, Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 24 (2006) 465–467.
[28] D.Y. Zhang, Z.P. Xu, H. Chiang, D.Q. Lu, Q.L. Zeng, Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells, Zhonghua Yu Fang Yi Xue Za Zhi 40 (2006) 149–152.
[29] R.J. Aitken, L.E. Bennetts, D. Sawyer, A.M. Wiklendt, B.V. King, Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline, Int. J. Androl. 28 (2005) 171–179.
[30] O. Erogul, E. Oztas, I. Yildirim, T. Kir, E. Aydur, G. Komesli, H.C. Irkilata, M.K. Irmak, A.F. Peker, Effects of electromagnetic radiation from a cellular phone on human sperm motility: an in vitro study, Arch. Med. Res. 37 (2006) 840–843.
[31] J.G. Yan, M. Agresti, T. Bruce, Y.H. Yan, A. Granlund, H.S. Matloub, Effects of cellular phone emissions on sperm motility in rats, Fertil. Steril. 88 (2007) 957–964.
[32] A. Agarwal, F. Deepinder, R.K. Sharma, G. Ranga, J. Li, Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study, Fertil. Steril. 89 (2008) 124–128.
[33] I. Fejes, Z. Za Vaczki, J. Szollosi, R.S. Kolosza, J. Daru, C.L. Kova, L.A. Pa, Is there a relationship between cell phone use and semen quality? Arch. Androl. 51 (2005) 385–393.
[34] A. Wdowiak, L. Wdowiak, H. Wiktor, Evaluation of the effect of using mobile phones on male fertility, Ann. Agric. Environ. Med. 14 (2007) 169–172.
[35] Z. Forgacs, Z. Somosy, G. Kubinyi, J. Bakos, A. Hudak, A. Surjan, G. Thuroczy, Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice, Reprod. Toxicol. 22 (2006) 111–117.
[36] M. Ozguner, A. Koyu, G. Cesur, M. Ural, F. Ozguner, A. Gokcimen, N. Delibas, Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field, Saudi Med. J. 26 (2005) 405–410.
[37] J.L. Roti Roti, R.S. Malyapa, K.S. Bisht, E.W. Ahern, E.G. Moros, W.F. Pickard, W.L. Straube, Neoplastic transformation in C3H 10T(1/2) cells after exposure to 835.62 MHz FDMA and 847.74 MHz CDMA radiations, Radiat. Res. 155 (2001) 239–247.
[38] R.S. Malyapa, E.W. Ahern, W.L. Straube, E.G. Moros, W.F. Pickard, J.L. Roti Roti, Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation, Radiat. Res. 1481 (1997) 608–617.
[39] R.S. Malyapa, E.W. Ahern, W.L. Straube, E.G. Moros, W.F. Pickard, J.L. Roti Roti, Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz), Radiat. Res. 148 (1997) 618–627.
[40] R.S. Malyapa, C. Bi, E.W. Ahern, J.L. Roti Roti, Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation, Radiat. Res. 149 (1998) 396–400.
[41] L. Li, K.S. Bisht, I. LaGroye, P. Zhang, W.L. Straube, E.G. Moros, J.L. Roti Roti, Measurement of DNA damage in mammalian cells exposed in vitro to radiofrequency fields at sars of 3–5 W/kg, Radiat. Res. 156 (2001) 328–332.
[42] G.J. Hook, P. Zhang, I. Lagroye, L. Li, R. Higashikubo, E.G. Moros, W.L. Straube, W.F. Pickard, J.D. Baty, J.L. Roti Roti, Measurement of DNA damage and apoptosis in molt-4 cells after in vitro exposure to radiofrequency radiation, Radiat. Res. 161 (2004) 193–200.
[43] I. Lagroye, R. R.Anane, B.A. Wettring, E.G. E.G.Moros, W.L. Straube, M. Laregina, M. Nichoff, W.F. Pickard, J. Baty, J.L. Roti, Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods, Int. J. Radiat. Biol. 80 (2004) 11–20.
[44] I. Lagroye, G.J. Hook, B.A. Wettring, J.D. Baty, E.G. Moros, W.L. Straube, J.L. Roti Roti, Measurements of alkali-labile DNA damage and protein-DNA crosslinks after 2450 MHz microwave and low-dose gamma irradiation in vitro, Radiat. Res. 161 (2004) 201–214.
[45] Vijayalaxmi, B.Z. Leal, M. Szilagyi, T.J. Prihoda, M.L. Meltz, Primary DNA damage in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation, Radiat. Res. 153 (2000) 479–486.
[46] R.R. Tice, G.G. Hook, M. Donner, D.I. McRee, A.W. Guy, Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells, Bioelectromagnetics 23 (2002) 113–126.
[47] J.P. McNamee, P.V. Bellier, G.B. Gajda, S.M. Miller, E.P. Lemay, B.F. Lavallee, L. Marro, A. Thansandote, DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field, Radiat. Res. 158 (2002) 523–533.
[48] J.P. McNamee, P.V. Bellier, G.B. Gajda, B.F. Lavallee, E.P. Lemay, L. Marro, A. Thansandote, DNA damage in human leukocytes after acute in vitro exposure to a 1.9 GHz pulse-modulated radiofrequency field, Radiat. Res. 158 (2002) 534–537.

[49] J.P. McNamee, P.V. Bellier, G.B. Gajda, B.F. Lavallee, L. Marro, E. Lemay, A. Thansandote, No evidence for genotoxic effects from 24 h exposure of human leukocytes to 1.9 GHz radiofrequency fields, Radiat. Res. 159 (2003) 693–697.

[50] O. Zeni, M. Romano, A. Perrotta, M.B. Lioi, R. Barbieri, G. d'Ambrosio, R. Massa, M.R. Scarfi, Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields, Bioelectromagnetics 26 (2005) 258–265.

[51] N. Sakuma, Y. Komatsubara, H. Takeda, H. Hirose, M. Sekijima, T. Nojima, J. Miyakoshi, DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations, Bioelectromagnetics 27 (2006) 51–57.

[52] L. Stronati, A. Testa, J. Moquet, A. Edwards, E. Cordelli, P. Villan, C. Marino, A.M. Fresegna, M. Appolloni, D. Lloyd, 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes, Int. J. Radiat. Biol. 82 (2006) 339–346.

[53] L. Verschaeve, P. Heikkinen, G. Verheyen, U. Van Gorp, F. Boonen, F. Vander Plaetse, A. Maes, T. Kumlin, J. Maki-Paakkanen, L. Puranen, J. Juutilainen, Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo, Radiat. Res. 165 (2006) 598–607.

[54] Y.R. Ahuja, B. Vijayashree, R. Saran, E.L. Jayashri, J.K. Manoranjani, S.C. Bhargava, In vitro effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay, Indian J. Biochem. Biophys. 36 (1999) 318–322.

[55] J. Delimaris, S. Tsilimigaki, N. Messini-Nicolaki, E. Ziros, S.M. Piperakis, Effects of pulsed electric fields on DNA of human lymphocytes, Cell Biol. Toxicol. 22 (2006) 409–415.

[56] R. Hong, Y. Zhang, Y. Liu, E.Q. Weng, Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice, Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23 (2005) 414–417.

[57] S. Ivancsits, E. Diem, A. Pilger, H.W. Rudiger, O. Jahn, Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts, Mutat. Res. 519 (2002) 1–13.

[58] S. Ivancsits, E. Diem, O. Jahn, H.W. Rudiger, Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields, Mech. Ageing Dev. 124 (2003) 847–850.

[59] S. Ivancsits, A. Pilger, E. Diem, O. Jahn, H.W. Rudiger, Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields, Mutat. Res. 583 (2005) 184–188.

[60] J. Jajte, M. Zmyslony, J. Palus, E. Dziubaltowska, E. Rajkowska, Protective effect of melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes, Mutat. Res. 483 (2001) 57–64.

[61] H. Lai, N.P. Singh, Melatonin, N-tert-butyl-alpha-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells, J. Pineal. Res. 22 (1997) 152–162.

[62] H. Lai, N.P. Singh, Magnetic-field-induced DNA strand breaks in brain cells of the rat, Environ. Health Perspect. 112 (2004) 687–694.

[63] R. Lourencini da Silva, F. Albano, L.R. Lopes dos Santos, A.D. Tavares Jr., I. Felzenszwalb, The effect of electromagnetic field exposure on the formation of DNA lesions, Redox. Rep. 5 (2000) 299–301.

[64] C. Schmitz, E. Keller, T. Freuding, J. Silny, H. Korr, 50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice, Acta Neuropathol. (Berl) 107 (2004) 257–264.

[65] B.M. Svedenstal, K.J. Johanson, K.H. Mild, DNA damage induced in brain cells of CBA mice exposed to magnetic fields, In Vivo 13 (1999) 551–552.

[66] R. Winker, S. Ivancsits, A. Pilger, F. Adlkofer, H.W. Rudiger, Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields, Mutat. Res. 585 (2005) 43–49.

[67] F.I. Wolf, A. Torsello, B. Tedesco, S. Fasanella, A. Boninsegna, M. D'Ascenzo, C. Grassi, G.B. Azzena, A. Cittadini, 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism, Biochim. Biophys. Acta 1743 (2005) 120–129.

[68] B. Yokus, D.U. Cakir, M.Z. Akdag, C. Sert, N. Mete, Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields, Free Radic. Res. 39 (2005) 317–323.

[69] M. Zmyslony, J. Palus, J. Jajte, E. Dziubaltowska, E. Rajkowska, DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz), Mutat. Res. 453 (2000) 89–96.

[70] K. Chow, W.L. Tung, Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis, FEBS Lett. 478 (2000) 133–136.

[71] J.G. Robison, A.R. Pendleton, K.O. Monson, B.K. Murray, K.L. O'Neill, Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure, Bioelectromagnetics 23 (2002) 106–112.

[72] H. Lai, Neurological effects of radiofrequency electromagnetic radiation, in: Mobile Phones and Health, Symposium, October 25–28, University of Vienna, Austria, 1998, see: http://www.waveguide.org/library/lai.html.

[73] A. Höytö, J. Juutilainen, J. Naarala, Ornithine decarboxylase activity is affected in primary astrocytes but not in secondary cell lines exposed to 872 MHz RF radiation, Int. J. Radiat. Biol. 83 (2007) 367–374.

[74] L.E. Anderson, J.E. Morris, L.B. Sasser, W. Löscher, Effects of 50- or 60-hertz, 100 microT magnetic field exposure in the DMBA mammary cancer model in Sprague-Dawley rats: possible explanations for different results from two laboratories, Environ. Health Perspect. 108 (2000) 797–802.

[75] A.L. Di Carlo, T.A. Litovitz, Is genetics the unrecognized confounding factor in bioelectromagnetics? Flock-dependence of field-induced anoxia protection in chick embryos, Bioelectrochem. Bioenerg. 48 (1999) 209–215.

[76] J.P. McNamee, J.R. McLean, C.L. Ferrarotto, P.V. Bellier, Comet assay: rapid processing of multiple samples, Mutat. Res. 466 (2000) 63–69.

[77] B. Oral, M. Guney, F. Ozguner, N. Karahan, T. Mungan, S. Comlekci, G. Cesur, Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C, Adv. Ther. 23 (2006) 957–973.

[78] M. Simkó, Cell type specific redox status is responsible for diverse electromagnetic field effects, Curr. Med. Chem. 14 (2007) 1141–1152.

[79] M. Blank, L. Soo, Electromagnetic acceleration of the Belousov-Zhabotinski reaction, Bioelectrochemistry 61 (2003) 93–97.

[80] M. Blank, R. Goodman, A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation, J. Cell. Physiol. 214 (2008) 20–26.

[81] K.R. Foster, R. Glaser, Thermal mechanisms of interaction of radiofrequency energy with biological systems with relevance to exposure guidelines, Health Phys. 92 (2007) 609–620.

[82] A. Peter, T. Valberg, E. van Deventer, M.H. Repacholi, Workgroup Report: Base Stations and Wireless Networks—Radiofrequency (RF) Exposures and Health Consequences, Environ. Health Perspect. 115 (2007) 416–424.

[83] D. Krewski, B.W. Glickman, R.W. Habash, B. Habbick, W.G. Lotz, R. Mandeville, F.S. Prato, T. Salem, D.F. Weaver, Recent advances in research on radiofrequency fields and health: 2001–2003, J. Toxicol. Environ. Health, Part B 10 (2007) 287–318.

[84] L. Hardell, M. Carlberg, F. Soderqvist, K.H. Mild, L.L. Morgan, Long-term use of cellular phones and brain tumors: increased risk associated with use for ≥10 years, Occup. Environ. Med. 64 (2007) 626–632.

[85] M. Mashevich, D. Folkman, A. Kesar, A. Barbul, R. Korenstein, E. Jerby, L. Avivi, Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability, Bioelectromagnetics 24 (2003) 82–90.

[86] W. Adey, C. Byus, C. Cain, R. Higgins, R. Jones, Spontaneous and nitrosourea-induced primary tumors of the central nervous system in Fischer 344 rats chronically exposed to 836 MHz modulated microwaves, Radiat. Res. 152 (1999) 293–302.
[87] J. Stammberger, W. Schmahl, L. Nice, The effects of X-irradiation, N-ethyl-N-nitrosourea or combined treatment on O^6-alkylguanine-DNA alkyltransferase activity in fetal rat brain and liver and the induction of CNS tumors, Carcinogenesis 11 (1990) 219–222.
[88] J.A. Rafferty, A.R. Clarke, D. Sellappan, M.S. Koref, I.M. Frayling, G.P. Margison, Induction of murine O6-alkylguanine-DNA-alkyltransferase in response to ionising radiation is p53 gene dose dependent, Oncogene 12 (1996) 693–697.
[89] T. Grombacher, U. Eichhorn, B. Kaina, p53 is involved in regulation of the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) by DNA damaging agents, Oncogene 17 (1998) 845–851.
[90] S. Krimsky, The weight of scientific evidence in policy and law, Am. J. Public Health 95 (2005) S129–S136.
[91] J. Juutilainen, S. Lang, Genotoxic, carcinogenic, and teratogenic effects of electromagnetic fields. Introduction and overview, Mutat. Res 387 (1997) 165–171.
[92] L. Verschaeve, A. Maes, Genetic, carcinogenic and teratogenic effects of radiofrequency fields, Mutat. Res. 410 (1998) 141–165.
[93] P. Gos, B. Eicher, J. Kohli, W.D. Heyer, No mutagenic or recombinogenic effects of mobile phone fields at 900 MHz detected in the yeast *saccharomyces cerevisiae*, Bioelectromagnetics 21 (2000) 515–523.
[94] J.J. Mond, N. Feuerstein, C.H. June, A.K. Balapure, R.I. Glazer, K. Witherspoon, M. Brunswick, Bimodal effect of phorbol ester on B cell activation, J. Biol. Chem. 266 (1991) 4458–4463.
[95] A. Kuzmin, J. Sandin, L. Terenius, S.O. Ogren, Dose- and time-dependent bimodal effects of k-opioid agonists on locomotor activity in mice, J. Pharmacol. Exp. Ther. 295 (2000) 1031–1042.
[96] J. Dierov, B.E. Sawaya, M. Prosniak, R.B. Gartenhaus, Retinoic acid modulates a bimodal effect on cell cycle progression in human adult T-cell leukemia cell, Clin. Cancer Res. 5 (1999) 2540–2547.
[97] R.E. Rosenstein, D.A. Golombek, B.I. Kanterewicz, D.P. Cardinali, Time-dependency for the bimodal effect of melatonin on calcium uptake in rat hypothalamus, J. Neurol. Trans. 85 (1991) 1435–1463.
[98] K.R. Popper, Conjectures and Refutations: The Growth of Scientific Knowledge, Harper & Row, NY, 1963.
[99] J. Siemiatycki, Synthesizing the lifetime history of smoking, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 2294–2295.
[100] M.J. Schoemaker, A.J. Swerdlow, A. Ahlbom, A. Auvinen, K.G. Blaasaas, E. Cardis, H.C. Christensen, M. Feychting, S.J. Hepworth, C. Johansen, L. Klaeboe, S. Lonn, P.A. McKinney, K. Muir, J. Raitanen, T. Salminen, J. Thomsen, T. Tynes, Mobile phone use and risk of acoustic neuroma: results of the Interphone case-control study in five North European countries, Brit. J. Cancer 93 (2005) 842–848.
[101] S. Sadetzki, A. Certrit, A. Jarus-Hakak, E. Cardis, Y. Deutch, S. Duvdevani, et al., Cellular phone use and risk of benign and malignant parotid gland tumors–a nationwide case-control study, Am. J. Epidemiol. 167 (4) (2008) 457–467
[102] A. Lahkola, A. Auvinen, J. Raitanen, M.J. Schoemaker, H.C. Christensen, M. Feychting, C. Johansen, L. Klaeboe, S. Lonn, A.J. Swerdlow, T. Tynes, T. Salminen, Mobile phone use and risk of glioma in 5 North European countries, Int. J. Cancer 120 (2007) 1769–1775.
[103] F. Oktem, F. Ozguner, H. Mollaoglu, A. Koyu, E. Uz, Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin, Arch. Med. Res. 36 (2005) 350–355.
[104] M. Yariktas, F. Doner, F. Ozguner, O. Gokalp, H. Dogru, N. Delibas, Nitric oxide level in the nasal and sinus mucosa after exposure to electromagnetic field, Otolaryngol. Head Neck Surg. 132 (2005) 713–716.
[105] F. Ozguner, Y. Bardak, S. Comlekci, Protective effects of melatonin and caffeic acid phenethyl ester against retinal oxidative stress in long-term use of mobile phone: a comparative study, Mol. Cell. Biochem. 282 (2006) 83–88.
[106] M. Porcelli, G. Cacciapuoti, S. Fusco, R. Massa, G. d'Ambrosio, C. Bertoldo, M. De Rosa, V. Zappia, Non-thermal effects of microwaves on proteins: thermophilic enzymes as model system, FEBS Lett. 402 (1997) 102–106.
[107] H. Bohr, J. Bohr, Microwave enhanced kinetics observed in ORD studies of a protein, Bioelectromagnetics 21 (2000) 68–72.
[108] F. Mancinelli, M. Caraglia, A. Abbruzzese, G. d'Ambrosio, R. Massa, E. Bismuto, Non-thermal effects of electromagnetic fields at mobile phone frequency on the refolding of an intracellular protein: myoglobin, J. Cell. Biochem. 93 (2004) 188–196
[109] M. Caraglia, M. Marra, F. Mancinelli, G. d'Ambrosio, R. Massa, A. Giordano, A. Budillon, A. Abbruzzese, E. Bismuto, Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells, J. Cell. Biochem. 204 (2005) 539–548.
[110] F. Crick, What Mad Pursuit: A Personal View of Scientific Discovery, Basic Books, New York, 1988.

ELSEVIER

Pathophysiology 16 (2009) 89–102

ISP
PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Genotoxic effects of radiofrequency electromagnetic fields

Hugo W. Ruediger *

Division of Occupational Medicine, Medical University of Vienna, Waehringer Guertel 18-20, Berggasse 4/33, 1090 Vienna, Austria

Received 24 October 2008; received in revised form 16 November 2008; accepted 16 November 2008

Abstract

101 publications are exploited which have studied genotoxicity of radiofrequency electromagnetic fields (RF-EMF) *in vivo* and *in vitro*. Of these 49 report a genotoxic effect and 42 do not. In addition, 8 studies failed to detect an influence on the genetic material, but showed that RF-EMF enhanced the genotoxic action of other chemical or physical agents. The controversial results may in part be explained by the different cellular systems. Moreover, inconsistencies may depend from the variety of analytical methods being used, which differ considerably with respect to sensitivity and specificity. Taking altogether there is ample evidence that RF-EMF can alter the genetic material of exposed cells *in vivo* and *in vitro* and in more than one way. This genotoxic action may be mediated by microthermal effects in cellular structures, formation of free radicals, or an interaction with DNA-repair mechanisms.

Keywords: Gene mutations; Cytogenetic effects; DNA fragmentation; Mechanisms of genotoxicity

1. Introduction

Alterations of genetic information in somatic cells are the key event in the process of carcinogenesis [1,2]. Consequently any agent, which has a genotoxic attribute is suspected also to be cancerogenic. This is the driving force behind the multitude of studies on genotoxicity of radiofrequency electromagnetic fields (RF-EMF), conducted so far. A total of 101 publications on genotoxicity studies of RF-EMF are exploited here, of which 49 report genotoxic effects, subsequently marked as GT(+) (Table 1), 43 do not (Table 2), and 9 find, that RF-EMF do not induce genotoxic events by itself but enhance the genotoxic action of other physical or chemical agents (Table 3). Thus, in contrast to several reviews in the past [3–6], it now became evident that non-thermal genotoxic effects of RF-EMF is convincingly demonstrated by a substantial number of published studies. The studies have been performed with a variety of different test systems – some studies used more than one test system – which will be assigned here to the three principle endpoints of a genotoxic action: (1) effect on chromosomes, (2) DNA fragmentation, and (3) gene mutations.

* Tel.: +43 1 9582908.
E-mail address: hugo.ruediger@meduniwien.ac.at.

0928-4680/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2008.11.004

2. Effect on chromosomes

This group comprises the analysis of numerical or structural anomalies of metaphase chromosomes (CA), sister-chromatid-exchanges (SCEs), and formation of micronuclei (MN). Of the 21 studies using CA, 9 are CA-positive, 11 CA-negative, and 1 reports an RF-induced enhancement of genotoxicity by X-rays. In general proliferating cells are required for the study of chromosomal effects, however, micronuclei have also been analysed in polychromatic erythrocytes and in exfoliated cells, for instance from buccal smears [7,8]. Moreover, aneuploidy rates of distinct chromosomes as well as chromosomal translocations can also be studied in interphase nuclei using fluorescence in situ hybridization (FISH). While structural aberrations detected by conventional CA are mainly lethal to the cell, translocations are persistent and may be passed to the cellular progeny. Using FISH increased levels of aneuploidy of chromosome 1, 10, 11, and 17 have been reported in human blood lymphocytes after RF-EMF exposure [9]. In metaphase chromosomes FISH may increase the sensitivity of chromosomal analysis [10] but this has only once been used for RF-EMF studies [11].

CA brings about to detect a variety of chromosomal aberrations. In contrast, micronuclei originate only from acentric

Table 1
Publications which report RF-EMF related genotoxic effects.

Reference	Biological system	Genotoxic endpoint	Results and comments
Aitken et al. [45]	Mouse sperm	QPCR and comet assay	Gel electrophoresis revealed no gross evidence of increased single- or double-DNA strand breakage in spermatozoa. However, a detailed analysis of DNA integrity using QPCR revealed damage to both the mitochondrial genome ($p < 0.05$) and the nuclear-globin locus ($p < 0.01$).
Balode [46]	Cow erythrocytes	Micronuclei (MN)	The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ($p < 0.01$) differences were found in the frequency distribution between the control and exposed groups.
Belyaev et al. [47]	Human blood lymphocytes	Chromatin condensation and 53BP1 foci	Decrease in background levels of 53BP1 foci and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation.
Busljeta et al. [48]	Rat hematopoietic tissues	MN	Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood (days 8 and 15). Concurrently, anuclear cells and erythropoietic precursor cells were decreased ($p < 0.05$) in the bone marrow on day 15, but micronucleated cells' (MNCs) frequency was increased.
d'Ambrosio et al. [49]	Human blood lymphocytes	MN	The micronucleus frequency was not affected by CW exposure; however, a statistically significant micronucleus effect was found following exposure to phase modulated field.
Diem et al. [23]	Human cultured fibroblasts and rat granulosa cells	Alkaline and neutral comet assay	The intermittent exposure showed a stronger effect in the comet assay than continuous exposure.
Ferreira et al. [50]	Rat hematopoietic tissues exposed during embryogenesis	MN	The irradiated group showed a significant increase in MN occurrence.
Fucic et al. [15]	Human blood lymphocytes	MN	X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.
Gadhia et al. [51]	Human blood lymphocytes	Chromosomal aberrations and SCE	There was a significant increase ($p < 0.05$) in dicentric chromosomes among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic. Synergistic action with MMC, SCEs showed a significant increase among mobile users.
Gandhi and Singh [7]	Human blood lymphocytes and buccal mucosa cells	Chromosomal aberrations and MN	Increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.
Gandhi, 2005 [52]	Human blood lymphocytes	Comet assay, *in vivo* capillary MN	Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The *in vivo* capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated cells.
Garaj-Vrhovac et al [53]	Human blood lymphocytes	Chromosomal aberrations and MN	In all experimental conditions, the frequency of all types of chromosomal aberrations was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples.
Garaj-Vrhovac et al. [54]	Chinese hamster cells V79	DNA synthesis by [3H]thymidine uptake, and chromosomal aberrations	In comparison with the control samples there was a higher frequency of specific chromosome lesions in cells that had been irradiated.
Garaj-Vrhovac et al. [55]	Chinese hamster cells V79	Chromosomal aberrations and MN	Significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells. The presence of micronuclei in irradiated cells confirmed the changes that had occurred in chromosome structure.
Garaj-Vrhovac et al. [56]	Human blood lymphocytes	MN	Increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.
Haider et al. [57]	*Tradescantia* flower buds	MN	The results at all exposure sites except one were statistically significant.
Koyama et al. [12]	CHO-K1 cells	MN + kinetochore determination	RF at SAR of 78 W/kg and higher form MN with a particular increase of kinetochore-positive MN and potentiate MN formation induced by bleomycine treatment.
Lai et al. [58]	Rat brain cells	Comet assay	RFR exposure significantly increased DNA double strand breaks in brain cells of the rat, and the effect was partially blocked by treatment with naltrexone.
Lai and Singh [59]	Rat brain cells	Alkaline comet assay	No effects immediately after 2 h of exposure to pulsed microwaves, whereas a dose rate-dependent increase in DNA single strand breaks was found in brain cells of rats at 4 h post-exposure with CW and pulsed waves.

Lai and Singh [60]	Rat brain cells	Comet assay	Significantly higher levels of DNA single and double strand breaks. Exposure to 'noise' alone did not significantly affect the levels, however, simultaneous 'noise' exposure blocked microwave-induced increases in DNA strand breaks.
Lai and Singh [61]	Rat brain cells	Comet assay	An increase in DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, no significant difference was observed between the effects of the two forms of radiation.
Lai and Singh [35]	Rat brain cells	Comet assay	Treatment immediately before and after RFR exposure with either melatonin or *N*-tert-butyl-alpha-phenylnitrone (PBN) blocks induction of DSB by RFR. It is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats.
Lixia et al. [62]	Human lens epithelial cells	Comet assay and BudR incorporation	No DNA breaks at 1 and 2 W/kg but increase 0 and 30 min after exposure to 3 W/kg. Exposure at 2 and 3 W/kg for 2 h significantly increased HsP 70 protein but not mRNA expression.
Maes et al. [63]	Human blood lymphocytes	Chromosome aberrations	Some cytogenetic damage was obtained *in vitro* when blood samples were very close to the antenna. The questionable *in vivo* results (six maintenance workers) are not considered here.
Maes et al. [64]	Human blood lymphocytes	Chromosomal aberrations, SCE, and MN	Marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and 19 micronuclei. On the other hand, the microwave exposure did not influence the cell kinetics nor the sister-chromatid-exchange (SCE) frequency.
Markova et al. [65]	Human blood lymphocytes	p53 binding protein and γH2AX foci	MWs from GSM mobile telephones affect chromatin conformation and 53BP1/gamma-H2AX foci similar to heat shock.
Mashevich et al. [66]	Human blood lymphocytes	Chromosomal aberrations	A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value.
Mazor et al. [9]	Human blood lymphocytes	Aneuploidy rate of Chr. # 1, 10, 11, 17 determined by interphase FISH	Increased levels of aneuploidy in chromosomes 1 and 10 at higher SAR, while for chromosomes 11 and 17 the increases were observed only for the lower SAR.
Nikolova et al. [67]	Mouse nestin-positive neural progenitor cells	Transcript of specific genes and proteins, proliferation, apoptosis, DNA DSB	Down-regulation of neural-specific Nurr1 and up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double strand breaks.
Paulraj and Behari [68]	Rat brain cells	Comet assay	Statistically significant ($p < 0.001$) increase in DNA single strand breaks in brain cells of rat.
Pavicic and Trosic [13]	V79 cells	Alteration of microtubule proteins	The microtubule structure altered after 3 h of irritation.
Phillips et al. [69]	Molt-4 T-lymphoblastoid cells	Comet assay	DNA damage decreased by (1) exposure to the iDEN signal (2.4 μW/g for 2 h or 21 h), (2) exposure to the TDMA signal (2.6 μW/g for 2 h and 21 h), (3) exposure to the TDMA signal (26 μW/g for 2 h), exposure to the iDEN signal (24 μW/g for 2 h) and 21 h significantly increased DNA damage.
Sarimov et al. [70]	Human blood lymphocytes	Chromatin condensation by anomalous viscosity	Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MW. Effects differ at various GSM frequencies and vary between donors.
Sarkar et al. [71]	Mouse testis and brain cells	Restriction pattern after HinfI treatment	As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7–8 kb which was also substantiated by densitometric analysis.
Schwarz et al. [33]	Human cultured fibroblasts and lymphocytes	Alkaline comet assay and MN	UMTS exposure increased the CTF and induced centromere-negative micronuclei in human cultured fibroblasts in a dose- and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin.
Sykes et al. [22]	pKZ1 mice	lacZ transgene inversion	No difference between the control and treated groups in the 1- and 5-day exposure groups, but a reduction in inversions below the spontaneous frequency in the 25-day exposure group. This suggests that RF radiation can lead to a perturbation in recombination frequency.
Tice et al. [72]	Human blood lymphocytes	Alkaline comet assay and MN	Exposure for either 3 or 24 h with the unmodulated signal did not induce a significant increase in DNA DSB or MN in lymphocytes. However, with the modulated signal there was a significant and reproducible increase in the frequency of micronucleated lymphocytes.
Tkalec et al. [14]	*Allium cepa* seeds	Germination, mitotic index, mitotic abnormalities	Increased mitotic aberrations in root meristematic cells of *A. cepa*. Effects were markedly dependent on the field frequencies applied as well as on field strength and modulation. Findings also indicate that mitotic effects of RF-EMF could be due to impairment of the mitotic spindle.
Trosic et al. [73]	Rat hematopoietic tissues	MN and polychromatic erythrocytes (PCEs)	The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ($p < 0.05$) in the subgroup exposed to fro/MW radiation after eight irradiation treatments of 2 h each in comparison with the sham-exposed control group.

Table 1 (*Continued*)

Reference	Biological system	Genotoxic endpoint	Results and comments
Trosic et al. [74]	Rat hematopoietic tissues	MN and polychromatic erythrocytes	In polychromatic erythrocytes significant differences ($p < 0.05$) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ($p < 0.05$).
Trosic and Busljeta [75]	Rat hematopoietic tissues and peripheral blood	MN and polychromatic erythrocytes	BMPCEs were increased on days 8 and 15, and PBPCEs were elevated on days 2 and 8 ($p < 0.05$).
Vijayalaxmi et al. [76]	C3H/Hel cancer prone mice, peripheral blood and bone marrow	MN	No observed RF effects. A correction was published, stating that there was actually a significant MN increase in peripheral blood and bone marrow cells after chronic exposure to RF [Vijayalaxmi, M.R. Frei, S.J. Dusch, V. Guel, M.L. Meltz, J.R. Jauchem, Radiat. Res. 149 (3) (1998) 308].
Wu et al. [39]	Human epithelial lens cells	Comet assay and intracellular ROS	RF at 4 W/kg for 24 h significantly increased intracellular ROS and DNA damage. Both can be blocked completely by electromagnetic noise.
Yadav and Sharma [8]	Exfoliated buccal cells	MN in buccal cells	In exposed subjects 9.84 ± 0.745 micronucleated cells and 10.72 ± 0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75 ± 0.774 MNC and 4.00 ± 0.808 TMN in controls. Correlation between 0–1, 1–2, 2–3 and 3–4 years of exposure and the frequency of MNC and TMN.
Yao et al. [40]	Human lens epithelial cells	Alkaline comet assay, gamma-H2AX foci, ROS level	SAR of 3 and 4 W/kg induced significant DNA damage in the comet assay, while no statistical difference in double strand breaks was found by γH2AX foci. Electromagnetic noise could block RF-induced ROS formation and DNA damage.
Yao et al. [41]	Human lens epithelial cells	Alkaline comet assay, γH2AX foci, ROS level	DNA damage was significantly increased by comet assay at 3 and 4 W/kg, whereas double strand breaks by γH2AX foci were significantly increased only at 4 W/kg. Significantly increased ROS levels were detected in the 3 and 4 W/kg groups.
Zhang et al. [77]	Chinese hamster lung cells (CHL)	γH2AX foci	Increased percentage of γH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 h ($37.9 \pm 8.6\%$) or 2-acetylaminofluorene exposure ($50.9 \pm 9.4\%$). However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 h ($31.8 \pm 8.7\%$).
Zotti-Martelli et al. [78]	Human blood lymphocytes	MN	Both spontaneous and induced MN frequencies varied in a highly significant way among donors ($p < 0.009$) and between experiments ($p < 0.002$), and a statistically significant increase of MN, although rather low, was observed dependent on exposure time ($p = 0.0004$) and applied power density ($p = 0.0166$).
Zotti-Martelli et al. [79]	Human blood lymphocytes	MN	The results showed for both radiation frequencies an induction of micronuclei as compared to the control cultures at a power density of 30 mW/cm^2 and after an exposure of 30 and 60 min.

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethansulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ1O), ethylmethansulfonate (EMS), chromosomal aberration analysis (CA), micronucleus assay (MN), reactive oxygen species (ROS), and fluorescence *in vitro* hybridization (FISH).

Table 2
Publications which do not report RF-EMF related genotoxic effects.

Reference	Biological system	Genotoxic endpoint	Results and comments
Antonopoulos et al. [80]	Human blood lymphocytes	SCE	No increase in SCE or cell cycle progression found.
Belyaev et al. [81]	Rat brain, spleen, and thymus	Comet assay	GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells.
Bisht et al. [82]	Mouse C3H 10T cells	MN	CDMA (3.2 or 4.8 W/kg) or FDMA (3.2 or 5.1 W/kg) RF-EMF radiation for 3, 8, 16 or 24 h did not result in a significant increase either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.
Chang et al. [83]	*Escherichia coli* tester strain	Bacterial mutagenicity (Ames test)	No mutagenic or co-mutagenic effect with 4-NQ1O.
Ciaravino et al. [84]	CHO cells	SCE	Radiofrequency electromagnetic radiation (RF-EMF) did not change the number of SCEs that were induced by adriamycin.
Garson et al. [85]	Human blood lymphocytes	CA	No RF-EMF effect observed.
Gorlitz et al. [86]	B6C3F1 mice lymphocytes, erythrocytes, and keratinocytes	MN	No visible effect.
Gos et al. [87]	*Saccharomyces cerevisiae*	Mutation rates	No effects in fluctuation tests on forward mutation rates at CAN1, on the frequency of petite formation, on rates of intra-chromosomal deletion formation, or on rates of intra-genic recombination in the absence or presence of MMS.
Hook et al. [88]	Molt-4 T lymphoblastoid cells	Comet assay	No RF-EMF effects observed.
Juutilainen et al. [89]	Female CBA/S mice and K2 female transgenic mice	MN in erythrocytes	No effect on MN frequency.
Kerbacher et al. [90]	CHO cells	CA	No alteration was observed in the extent of chromosome aberrations induced by either simultaneous fro radiation exposure or convection heating to equivalent temperatures.
Komatsubara et al. [91]	Mouse m5S cells	CA	No effect on CA; temperature increase up to 41 °C at 100 W/kg.
Koyama et al. [92]	CHO cells	MN	No MN increase in cells exposed to HFEMF at a SAR of lower than 50 W/kg, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls (temperature effect).
Lagroye et al. [93]	Rat brain cells	Alkaline comet assay	No observed effect.
Lagroye et al. [94]	C3H 10T1/2 cells	Comet assay, DNA–protein crosslinks	No observed effect.
Li et al. [95]	Murine C3H 10T cells	Comet assay	No observed effect.
Maes et al. [96]	Human blood lymphocytes	CA, SCE	Combined exposure of RF-EMF and to MMC and X-rays. Overall, no indication was found of a mutagenic, and/or co-mutagenic/synergistic effect.
Maes et al. [97]	Human blood lymphocytes	CA, SCE	Combined treatments with X-rays or MMC did not provide any indication of a synergistic action between the RF-EMF fields and X-rays or MMC.
Maes et al. [98]	Human blood lymphocytes	CA, SCE, Comet assay	The alkaline comet assay, SCE, and CA tests revealed no evidence of RF-EMF-induced genetic effects. No cooperative action was found between the electromagnetic field exposure and MMC using either the comet assay or SCE test.
Malyapa et al. [99]	Rat brain cells	Comet assay	No significant differences observed.
Malyapa et al. [100]	U87MG and C3H 10T1/2 cells	Comet assay	No significant differences observed.
Malyapa et al. [101]	U87MG and C3H 10T1/2 cells	Comet assay	No significant differences observed.
McNamee et al. [102]	Human blood lymphocytes	Comet assay and MN	No significant differences observed.
McNamee et al. [103]	Human blood lymphocytes	Comet assay and MN	No significant differences observed.
McNamee et al. [104]	Human blood lymphocytes	Comet assay	No significant differences observed.
Meltz et al. [105]	L5178Y mouse leukemic cells	Mutation in TK locus	No effect of RF-EMF alone or in the induced mutant frequency due to the simultaneous exposure to RF-EMF and proclaim, as compared with the proflavin exposures alone.
Ono et al. [106]	lacZ-transgenic mice	Mutations at the lac gene in spleen, liver, brain and testis	Mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were similar to those observed in non-exposed mice.

Table 2 (*Continued*)

Reference	Biological system	Genotoxic endpoint	Results and comments
Roti Roti et al. [107]	C3H 10T1/2 cells	Transformed foci	No statistically significant differences observed.
Sakuma et al. [108]	Human glioblastoma A172 cells and fetal lung fibroblasts	DNA strand breaks (comet assay?)	No statistically significant differences.
Scarfi et al. [109]	Human blood lymphocytes	MN	No statistically significant differences observed.
Speit et al. [24]	Human cultured fibroblasts	Comet assay and MN	No statistically significant differences observed.
Stronati et al. [110]	Human blood lymphocytes	Comet assay, CA, SCE, MN	By comparison with appropriate sham-exposed and control samples, no effect of RF-EMF alone could be found for any of the assay endpoints. In addition RF-EMF did not modify any measured effects of the X-radiation.
Takahashi et al. [111]	Big Blue mice brain tissues	lacZ transgene inversion	No statistically significant differences observed.
Verschaeve et al. [112]	Rat brain and liver tissues, erythrocytes	MN (erythrocytes) and comet assay	No genotoxic effect of RF-EMF alone. Co-exposures to MX and RF-EMF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only.
Vijayalaxmi et al. [113]	Human blood lymphocytes	CA and MN	No observed RF-EMF effects.
Vijayalaxmi et al. [114]	Human blood lymphocytes	CA and MN	No observed RF-EMF effects.
Vijayalaxmi et al. [115]	Human blood lymphocytes	Comet assay	No observed RF-EMF effects.
Vijayalaxmi et al. [116]	Human blood lymphocytes	CA, MN	No observed RF-EMF effects.
Vijayalaxmi et al. [117]	Rat hematopoietic tissues and erythrocytes	MN	No observed RF-EMF effects.
Vijayalaxmi et al. [118]	Rat whole body and head only exposures. BM erythrocytes	MN	No observed RF-EMF effects.
Vijayalaxmi et al. [119]	CF-1 male mice, peripheral blood and bone marrow	MN	No observed RF-EMF effects.
Zeni et al. [120]	Human blood lymphocytes	Comet assay, CA, SCE	No observed RF-EMF effects.
Zeni et al. [121]	Human blood lymphocytes	MN	No observed RF-EMF effects.

Abbreviations: Chromosomal aberration analysis (CA), methotrexat (MX), mitomycin C (MMC), 4-nitroqinoline-1-oxide (4-NQ1O), methylmethansulfonate (MMS), code division multiple access (CDMA), frequency division multiple access (FDMA), and time division multiple access (TDMA).

Table 3
Publications which report synergistic RF-EMF effects in combination with other genotoxicants.

Reference	Genotoxic agents	Biological system	Genotoxic endpoint	Results and comments
Baohong et al. [122]	MMC, BLM, MMS, 4-NQ1O	Human blood lymphocytes	Alkaline comet assay	1.8 GHz RFR (SAR, 3 W/kg) for 2 h did not induce DSB, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4-NQ1O. The synergistic DNA damage effects with BLM or MMS were not obvious.
Baohong et al. [123]	254 nm UVC	Human blood lymphocytes	Alkaline comet assay	RF exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5 and 4 h incubation respectively.
Kim et al. [124]	Cyclophosphamide, 4-NQ1O, EMS	L5178Y mouse lymphoma cells (comet assay) and CHL cells (CA)	Alkaline comet assay and CA	No direct cytogenetic effect of RF alone or in combination with cyclophosphamide or 4-NQ1O was found in the CA test and in the comet assay. However, RF had a potentiating effect in combination with cyclophosphamide or 4-NQ1O.
Maes et al. [125]	MMC	Human blood lymphocytes	SCE	Synergistic effect was observed with MMC.
Maes et al. [126]	MMC	Human blood lymphocytes	CA, SCE, comet assay	The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.
Manti et al. [11]	Previous 4 Gy X-ray radiation	Human blood lymphocytes	Chromosome aberration by FISH	No significant variations due to the UMTS exposure in the fraction of aberrant cells, but frequency of exchanges per cell in X-ray irradiated cells was significantly increased by UMTS at 2 W/kg.
Wang et al. [127]	254 nm UVC	Human blood lymphocytes	Comet assay	RF did not induce DNA damage but reduced or enhanced DNA damage by UVC at 1.5 or 4.0 h respectively.
Wang et al. [128]	MMC, BLM, MMS, 4-NQ1O	Human blood lymphocytes	Comet assay	RF did not induce DNA damage but enhanced DNA damage induced by MMC and 4-NQ1O.
Zhang et al. [129]	MMC	Human blood lymphocytes	Comet assay, micronucleus assay	No RF-induced DNA and chromosome damage, but increased MMC DNA damage by RF in comet assay.

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethansulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ1O), ethylmethansulfonate (EMS), chromosomal aberration analysis (CA), fluorescence *in vitro* hybridization (FISH).

fragments of chromosomes or from lagged chromosomes secondary to mitotic non-disjunction, the latter being detected by indirect immunofluorescence using kinetochore antibodies. Kinetochore-positive MN arise by epigenetic mechanisms (disturbances of the spindle apparatus). Kinetochore-negative MN arise from acentric chromosomal fragments. This is an important distinction, but has been performed in a few RF-EMF studies only, of which only one [12] reports an increase of kinetochore-positive MN albeit after a high SAR ≥ 78 W/kg. Two studies describe RF-EMF-induced disturbances of the spindle apparatus [13,14], and one reports an aneugenic RF-EMF effect on the basis of the size distribution of MN [15]. Of a total of 39 studies using the micronucleus assay 22 are MN-positive, and 17 MN-negative.

SCEs are analysed in metaphase chromosomes after two rounds of replication in the presence of 5-bromodeoxyuridine (BUDR). SCEs, which are induced during the S-phase of the cell cycle, represent an exchange between homologous chromatids, an event which by itself is genetically neutral. Nevertheless it is considered to reflect a recombinational repair of DNA double strand breaks (DSB), and may therefore serve as an indicator of genotoxic stress. Of 10 studies using SCE a GT(+) effect was reported in one only, 8 were negative, and one study reports RF-induced enhancement of genotoxicity by mitomycin C.

3. DNA fragmentation

The comet assay, also known as a "Single Cell Gel electrophoresis assay" (SCG), and the detection of gamma-H2AX foci are the most frequently used techniques to study RF-EMF-induced DNA strand breaks. The comet assay uses interphase nuclear DNA, which is unwinded under alkaline conditions and subsequently subjected to an electric field. Here DNA fragments migrate towards the anode, thereby forming a comet-like tail [16,17]. The alkaline comet assay detects DNA single strand as well as double strand breaks, but is not applicable in the presence of DNA crosslinking agents [18]. These breaks may occur not only by toxic influences but also by transcriptional and repair processes and by alkali-sensitive sites. Therefore this frequently used and very sensitive assay has a poor specificity. Of 41 studies using the comet assay 15 report comet-positive and 19 comet-negative results after RF-EMF exposure. RF-EMF enhancement of comet assay effects caused by other genotoxic agents is described in 7 studies.

Out of a multitude of DNA damage checkpoint proteins two have been used to detect DBS: H2AX, a member of the nuclear histone family [19], and P53 binding protein (53BP1). Both are rapidly phosphorylated only minutes after DNA damage and are then gathered in the vicinity of DNA double strand breaks. Here they form foci which can be visualized by indirect immunofluorescence [20,21]. These foci represent an initial and specific step in the repair process of exogenously induced DNA double strand breaks. It is important to realize, however, that repair processes of DSB are quantified, not DSB themselves. The method has been employed in 4 studies, predominantly using the γH2AX foci test. In all instances GT(+) effects have been detected.

DNA alterations have also been analysed by the anomalous viscosity time dependency test (AVTD, 1 GT(+) study), detecting conformational changes, and by quantitative PCR (QPCR, 1 GT(+) study) detecting structural changes in the DNA.

4. Gene mutations

In this category 6 studies have been performed using 4 different endpoints: (1) Altered restriction fragments (1 GT(+) study), (2) lacZ inversion in transgenic mice. This method has been used in 3 studies which all failed to detect an increased rate of inversions, but one found a reduced rate as compared to unexposed controls [22], which is interpreted as a RF-EMF-induced reduction of recombination repair. (3) Mutation at the thymidine kinase (TK) locus (1 negative study). (4) Bacterial his^- revertants (Ames test, 1 negative study).

5. Discussion

The large number of contradictory results among the 101 published studies on a genotoxic action of RF-EMF is tangling. Nevertheless patterns can be perceived. GT(+) as well as GT(−) findings have been reported at a standard absorption ratio (SAR) below 0.05 up to 100 W/kg and an exposure of 15 min and 48 h *in vitro*, and between hours and years *in vivo*. The outcome of studies was nearly independent from RF frequencies between 300 and 7700 MHz and the type of RF signal, either continuous wave (CW) or pulse-modulated (PM). GT(+) was obtained in 15 CW and 26 PM exposures, GT(−) in 14 CW and 27 PM exposures (some studies did not indicate the type of signal used). Contradictory results have been obtained even when two experienced groups performed the same experiments using the same cells and identical exposure conditions [23,24]. This may reflect a general problem of genotoxic studies being dependent on a multitude of factors which are difficult to control [25]. Some of the studies exploited here have shortcomings with respect to incompletely described or unreliable exposure conditions and/or an inadequate experimental design. Even a considerable publication bias in favour of negative results has been suspected (www.microwavenews.com/RR.html, 2006) [26].

The proportion of GT(+) effects is much higher *in vivo* (23/40) than *in vitro* (29/77). (Since some studies have been performed on more than one biological system, the total number of GT(+) and GT(−) effects exceeds the total number of published studies.) Considering all genotoxic endpoints applied, the frequently used parameters chromosome analysis (9/21 GT(+)), comet assay (15/41 GT(+)), and sister-chromatid-exchange (1/10 GT(+)) showed the highest

proportion of negative results, while the micronucleus assay yielded more positive than negative results (22/39 GT(+)). Since the SCE test which was negative in nearly all cases is known to be rather insensitive to radiomimetic (clastogenic) agents it can be speculated, that a clastogenic mechanism is involved in RF-EMF genotoxic action.

Epigenetic influences may also contribute to genotoxicity as demonstrated by RF-EMF-induced chromosomal non-disjunction and disturbances of the mitotic spindle. This is in agreement with the higher proportion of 22/39 GT(+) findings among studies using the micronucleus assay as compared to those using CA, because some of the micronuclei may represent lagged chromosomes. Epigenetic mechanisms may also be effective after a combined exposure to RF-EMF and various physical or chemical mutagens (Table 4). RF-EMF preferentially enhanced the genotoxic effect of 4-NQ1O (4/4), MMC (4/8), UVC (2/2), and cyclophosphamide (2/2). No synergistic effect was obtained using MMS and EMS (3/3), BLM (2/2), and adriamycine (2/2). Only one out of 3 studies reported a synergistic effect with X-rays.

Cells and tissues of different origin exhibit a clearly variable sensitivity for genotoxic RF-EMF effects (Table 4). This has also been observed with extremely low frequency (ELF)-EMF [27] and may be dependent on genetic differences [28]. GT(+) effects of RF-EMF were reported predominantly in the following biological systems: human lens epithelial cells (4/4), human buccal mucosa cells (2/2), rodent brain tissues (8/13), and rat hemopoietic tissues (5/7). GT(−) results have been obtained with mouse permanent cell lines (7/7) and permanent lymphoblastoid cells of various origin (7/7). This is in a striking analogy to RF-EMF-induced reduction of ornithine decarboxylase activity being detected in primary but not in secondary neural cells [29].

6. Proposed mechanisms of RF-EMF genotoxicity

Cells are unusually sensitive to electromagnetic fields [30]. Weak fields may accelerate electron transfer and thereby destabilize the H-bond of cellular macromolecules. This could explain the stimulation of transcription and protein expression, which has been observed after RF-EMF exposure [31,32]. However, the energy of weak EM fields is not sufficient directly to break a chemical bond in DNA. Therefore it can be concluded, that genotoxic effects are mediated by indirect mechanisms as microthermal processes, generation of oxygen radicals (ROS), or a disturbance of DNA-repair processes.

6.1. Thermal effects

An increase of temperature in the culture medium of RF-EMF exposed cells has been observed at very high SAR levels only [12]. The vast majority of GT(+) studies were conducted at SAR < 2.0 not leading to a detectable increase of temperature in the culture medium. Moreover, similar or larger effects have been observed at a 5′ on/10′ off intermittent exposure [23,33], a result that contradicts a

Table 4
Distribution RF-EMF effects in 101 published studies.

Biological system	RF-EMF effects		Synergistic effects	
	Positive	Negative	Positive	Negative
In vitro (all cells and tissues)	29	39	9	11
Human blood lymphocytes	18	23	8	4
Human lens epithelial cells	4			
Human cultured fibroblasts	2	2		
Human glioblastoma cells		3		
Human lymphoblastoid cells		2		
Mouse permanent cell lines		6		1
Mouse lymphoblastoid cells		1	1	1
Chinese hamster cells (CHO, V79)	4	2		3
E. coli		1		2
Yeast		1		
Rat granulosa cells	1			
In vivo (all species and tissues)	23	17	0	1
Human blood lymphocytes	4	2		
Human buccal mucosa cells	2			
Mouse sperm	1			
Mouse brain tissues	2			
Mouse polychromatic erythrocytes		4		
Rat brain tissues	6	4		1
Rat hemopoietic tissues	5	2		
Rat spleen, liver		2		
lacZ-transgenic mice		3		
Plants	2			
Cattle polychromatic erythrocytes	1			

Since several published studies have used more than 1 biological system the total of negative and positive effects exceeds the number of 101 publications.

simple temperature-based mechanism of the observed genotoxic action. However, experimental results with microwave absorption at colloidal interfaces have demonstrated that the electric absorption of microwaves between 10 and 4000 MHz goes through a maximum with the size of bride droplets >100 and <10,000 nm, and depends on the type of ions and their concentrations [34]. This local absorption of microwaves may therefore lead to a considerable local heating in living cells during low energy microwave exposure.

6.2. Oxygen radicals

There is evidence that RF-EMF may stimulate the formation of reactive oxygen species in exposed cells *in vivo* [35–37] and *in vitro* [38–41]. Free oxygen radicals may form base adducts in DNA, the most important lesion being 8-OHdG, and oxidize also other cellular components, such as lipids leaving behind reactive species, that in turn can couple to DNA bases [42]. The first step in the generation of ROS by microwaves is mediated in the plasma membrane by NADH oxidase [43]. Subsequently ROS activates matrix metalloproteases (MMP), thereby initiating intracellular signalling cascades. It is interesting to note that these processes start within 5 min of radiation and at a very low field intensity of 0.005 W/cm^2. Moreover, higher effects have been obtained by intermittent radiation, when cells were left unirradiated for 10 min. This is in agreement with *in vitro* genotoxicity studies using the comet assay [23,33].

6.3. Alteration of DNA-repair processes

A considerable proportion of studies have investigated the consequences of a combined exposure to RF-EMF and various chemical or physical mutagens. 8/12 studies using human blood lymphocytes have demonstrated that RF-EMF enhanced the genotoxic action of other agents, preferentially of UV, MMC, or 4-NQ1O (an UV-mimetic agent). Since in all these experiments microwave exposure failed to induce detectable genotoxic effect by itself, an interference with DNA-repair mechanisms has been postulated, however, there is no direct experimental proof yet. An alteration of recombinational repair has also been proposed by Sykes et al. [22] as an explanation of the reduced rate of inversions in lacZ-transgenic mice after RF-EMF treatment.

An influence of microwave exposure on DNA-repair processes has long been proposed for power frequency electromagnetic fields [35]. A recent epidemiological investigation into the frequency of polymorphisms of DNA-repair genes in children with acute leukemia living in the vicinity of power line transformers [44] emphasizes the significance DNA-repair impairment for an EMF related increase of this malignancy. There was a significant gene–environment interaction (COR = 4.31) between the electromagnetic field intensities and a less active genetic variant of XRCC1, a crucial enzyme in base excision repair.

References

[1] B.N. Ames, W.E. Durston, E. Yamasaki, F.D. Lee, Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 2281–2285.

[2] W.F. Bodmer, Cancer genetics, Br. Med. Bull. 50 (1994) 517–526.

[3] L. Verschaeve, A. Maes, Genetic, cancerogenic and teratotogenic effects of radiofrequency fields, Mutat. Res. 410 (1998) 141–165.

[4] M.L. Meltz, Radiofrequency exposure and mammalian cell toxicity, genotoxicity, and transformation, Bioelectromagnetics (Suppl. 6) (2003) S196–S213.

[5] Vijayalaxmi, G. Obe, Controversial cytogenetic observations in mammalian somatic cells exposed to radiofrequency radiation, Radiat. Res. 162 (2004) 481–496.

[6] L. Verschaeve, Genetic effects of radiofrequency radiation (RFR), Toxicol. Appl. Pharmacol. 2007 (2005) S336–S341.

[7] G. Gandhi, P. Singh, Cytogenetic damage in mobile phone users: preliminary data, Int. J. Hum. Genet. 5 (4) (2005) 259–265.

[8] O. Yadav, M.K. Sharma, Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations, Mutat. Res. 650 (2008) 175–180.

[9] R. Mazor, A. Korenstein-Ilan, A. Barbul, Y. Eshet, A. Sahadi, E. Jerby, Korenstein, Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiafrequency electromagnetic fields for 72 hours, Radiat. Res 169 (2008) 28–37.

[10] M.R. Speicher, N.P. Carter, The new cytogenetics: Blurring the boundaries with molecular biology, Nature Rev. Genet. 6 (2005) 782–792.

[11] L. Manti, H. Braselmann, M.L. Calabrese, R. Massa, M. Pughese, P. Scampoli, G. Sicignano, G. Grossi, Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro, Radiat. Res. 169 (2008) 575–583.

[12] S. Koyama, T. Nakahara, K. Wake, M. Taki, Y. Isozumi, J. Miyakoshi, Effects of high frequency electromagnetic fields on micronucleus formation inCHO-K1 cells, Mutat. Res. 541 (2003) 81–89.

[13] I. Pavicic, I. Trosic, In vitro testing of cellular response to ultra high frequency electromagnetic field radiation, Toxicol. In Vitro 22 (2008) 1344–1348.

[14] M. Tkalec, K. Malaric, M. Pavlica, B. Pevalek-Kozlina, Z. Vidakovic-Cifrek, Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of *Allium cepa* L., Mutat. Res. (2008), doi:10.1016/j.mrgentox.2008.09.022.

[15] A. Fucic, V. Garaj-Vrhovac, M. Skara, B. Dimitrovic, X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes, Mutat. Res. 282 (4) (1992) 265–271.

[16] O. Ostling, K.J. Johanson, Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells, Biochem. Biophys. Res. Commun. 123 (1984) 291–298.

[17] N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.

[18] G. Speit, M. Fröhler-Keller, P. Schütz, S. Neuss, Low sensitivity of the comet assay to detect acetaldehyde-induced genotoxicity, Mutat. Res. 657 (2008) 93–97.

[19] T.T. Paull, E.P. Rogakou, V. Yamazaki, C.U. Kirchgessner, M. Gellert, W.M. Bonner, A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage, Curr. Biol. 10 (2000) 886–895.

[20] O. Fernandez-Capetillo, H.A.T. Chen, A. Celeste, I. Ward, P.J. Romanienko, J.C. Morales, K. Naka, Z. Xia, D. Camerini-Otero, N. Motoyama, P.B. Carpenter, W.M. Bonner, J. Chen, A. Nussenzweig, DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1, Nat. Cell Biol. 4 (2002) 993–997.

[21] O. Fernandez-Capetillo, A. Lee, M. Nussenzweig, A. Nussenzweig, H2AX: the histone guardian of the genome, DNA Repair 3 (2004) 959–967.
[22] P.J. Sykes, B.D. McCallum, M.J. Bangay, A.M. Hooker, A.A. Morley, Effect of exposure to 900 MHz radiofrequency radiation on intrachromosomal recombination in pKZ1 mice, Radiat. Res. 156 (5) (2001) 495–502.
[23] E. Diem, C. Schwarz, F. Adlkofer, O. Jahn, H.W. Ruediger, Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro, Mutat. Res. 583 (2005) 178–183.
[24] G. Speit, P. Schuetz, H. Hoffmann, Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible, Mutat. Res. 626 (2007) 42–47.
[25] D. Blakey, S.M. Galloway, D.J. Kirkland, J.T. MacGregor, Regulatory aspects of genotoxicity testing: from hazard identification to risk assessment, Mutat. Res. 657 (2008) 84–90.
[26] A. Huss, M. Egger, K. Hug, K. Huwiler-Müntener, M. Röösli, Source of funding and results of studies of health effects of mobile phone use: systematic review of experimental studies, Environ. Health. Perspect. 115 (2007) 1–4.
[27] S. Ivancsits, A. Pilger, E. Diem, O. Jahn, H.W. Rüdiger, Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields, Mutat. Res. 583 (2005) 184–188.
[28] A.L. Di Carlo, T.A. Litovitz, Is genetics the unrecognized confounding factor in bioelectromagnetics? Flock-dependence of field-induced anoxia protection in chick embryos, Bioelectrochem. Bioelectromagn. 48 (1999) 209–215.
[29] A. Höytö, J. Juutilainen, J. Naarala, Ornithine decarboxylase activity is affected in primary astrocytes but not in secondary cell lines exposed to 872 MHz RF radiation, Int. J. Radiat. Biol. 83 (6) (2007) 367–374.
[30] M. Blank, R. Goodman, Initial interactions in electromagnetic field-induced biosynthesis, J. Cell. Physiol. 199 (2004) 359–363.
[31] S. Lee, D. Johnson, K. Dunbar, H. Dong, X. Ge, Y.C. Kim, C. Wing, N. Jayathilaka, N. Emmanuel, C.Q. Zhou, H.L. Gerber, C.C. Tseng, S.M. Wang, 2.45 GHz radiofrequency fields alter gene expression in cultured human cells, FEBS Lett. 579 (21) (2005) 4829–4836.
[32] R. Nylund, D. Leszczynski, Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent, Proteomics 17 (2006) 4769–4780.
[33] C. Schwarz, E. Kratochvil, A. Pilger, N. Kuster, F. Adlkofer, H.W. Ruediger, Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes, Int. Arch. Occup. Environ. Health 81 (2008) 755–767.
[34] C. Holtze, R. Sivaramakrishnan, M. Antonietti, J. Tsuwi, F. Kremer, K.D. Kramer, The microwave absorption of emulsions containing aqueous micro- and nanodroplets: a means to optimize microwave heating, J. Colloid Interf. Sci. 302 (2) (2006) 651–657.
[35] H. Lai, N.P. Singh, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells, Bioelectromagnetics 18 (6) (1997) 446–454.
[36] F. Oktem, F. Ozguner, H. Mollaoglu, A. Koyu, E. Zu, Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin, Arch. Med. Res. 36 (4) (2005) 350–355.
[37] M. Tkalec, K. Malaric, B. Pevalek-Kozlina, Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L., Sci. Total Environ. 388 (1–3) (2007) 78–89.
[38] M. Zmyslony, P. Politanski, E. Rajkowska, W. Szymczak, J. Jajte, Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions, Bioelectromagnetics 25 (2004) 324–328.
[39] W. Wu, K. Yao, K.J. Wang, D.Q. Lu, J.L. He, L.H. Xu, W.J. Sun, Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields, Zhejiang Da Xue Xue Bao Yi Xue Ban 37 (2008) 34–38 (Chinese languange).
[40] K. Yao, W. Wu, K. Wang, S. Ni, P. Ye, Y. Ye, J. Ye, L. Sun, Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells, Mol. Vis. 19 (2008) 964–969.
[41] K. Yao, W. Wu, Y. Yu, Q. Zeng, J. He, D. Lu, K. Wang, Effect of superposed electromagnetic noise on lens epithelial cells induced by microwave radiation, Invest. Ophthalmol. Vis. Sci. 49 (2008) 2009–2015.
[42] M. Valko, M. Izakovic, M. Mazur, C.J. Rhodes, J. Telser, Role of oxygen radicals in DNA damage and cancer incidence, Mol. Cell. Biochem. 266 (2004) 37–56.
[43] J. Friedman, S. Kraus, Y. Hauptman, Y. Schiff, R. Seger, Mechanisms of short-term ERK activation by electromagnetic fields at mobile phone frequencies, Biochem. J. 405 (2007) 559–568.
[44] Y. Yang, J. Xingming, Y. Chonghuai, T. Ying, T. Jingyan, S. Xiaoming, Case-only study of interactions between DNA repair genes (hMLH1, APEX1, MGMT, XRCC1 and XPD) and low-frequency electromagnetic fields in childhood acute leukemia, Leuk. Lymphoma 49 (12) (2008) 2344–2350.
[45] R.J. Aitken, L.E. Bennetts, D. Sawyer, A.M. Wiklendt, B.V. King, Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline, Int. J. Androl. 28 (2005) 171–179.
[46] Z. Balode, Assessment of radio-frequency electromagnetic radiation by the micronucleus test in bovine peripheral erythrocytes, Sci. Total Environ. 180 (1) (1996) 81–85.
[47] I.Y. Belyaev, L. Hillert, M. Protopopova, C. Tamm, L.O. Malmgren, B.R. Persson, G. Selivanova, M. Harms-Ringdahl, 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons, Bioelectromagnetics 26 (3) (2005) 173–184.
[48] I. Busljeta, I. Trosic, S. Milkovic-Kraus, Erythropoietic changes in rats after 2.45 GJz nonthermal irradiation, Int. J. Hyg. Environ. Health 207 (6) (2004) 549–554.
[49] G. d'Ambrosio, R. Massa, M.R. Scarfi, O. Zeni, Cytogenetic damage in human lymphocytes following GMSK phase modulated microwave exposure, Bioelectromagnetics 23 (2002) 7–13.
[50] A.R. Ferreira, T. Knakievicz, M.A. Pasquali, D.P. Gelain, F. Dal-Pizzol, C.E. Fernandez, A.A. DeSalles, H.B. Ferreira, J.C. Moreira, Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring, Life Sci. 80 (2006) 43–50.
[51] P.K. Gadhia, T. Shah, A. Mistry, M. Pithawala, D. Tamakuwala, A preliminary study to assess possible chromosomal damage among users of digital mobile phones, Electromagn. Biol. Med. 22 (2–3) (2003) 149–159.
[52] G.A. Gandhi, Genetic damage in mobile phone users: some preliminary findings, Indian Hum. Genet. 11 (2) (2005) 99–104.
[53] V. Garaj-Vrhovac, A. Fucic, D. Horvat, The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro, Mutat. Res. 281 (3) (1992) 181–186.
[54] V. Garaj-Vrhovac, D. Horvat, Z. Koren, The effect of microwave radiation on the cell genome, Mutat. Res. 243 (2) (1990) 87–93.
[55] V. Garaj-Vrhovac, D. Horvat, Z. Koren, The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation, Mutat. Res. 263 (3) (1991) 143–149.
[56] V. Garaj-Vrhovac, Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation, Chemosphere 39 (13) (1999) 2301–2312.
[57] T. Haider, S. Knasmueller, M. Kundi, M. Haider, Clastogenic effects of radiofrequency radiations on chromosomes of *Tradescantia*, Mutat. Res. 324 (1–2) (1994) 65–68.